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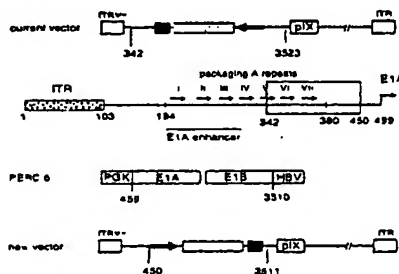
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(54) Title: ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIV1-GAG, POL, NEF AND MODIFICATIONS



Modifications made to the current adenovector backbone in the generation of the new vector.

(57) Abstract: First generation adenoviral vectors and associated recombinant adenovirus-based HIV vaccines which show enhanced stability and growth properties and greater cellular-mediated immunity are described within this specification. These adenoviral vectors are utilized to generate and produce through cell culture various adenovirus-based HIV-1 vaccines which contain HIV-1 gag, HIV-1 pol and/or HIV-1 nef polynucleotide pharmaceutical products, and biologically relevant modifications thereof. These adenovirus vaccines, when directly introduced into living vertebrate tissue, preferably a mammalian host such as a human or a non-human mammal of commercial or domestic veterinary importance, express the HIV-1 Gag, Pol and/or Nef protein or biologically modification thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding HIV-1 Gag, encoding codon optimized HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNase H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The adenovirus vaccines of the present invention, when administered alone or in a combined modality regime, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.



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## TITLE OF THE INVENTION

ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING  
CODON OPTIMIZED HIV1-GAG, POL, NEF AND MODIFICATIONS

## 5 CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit, under 35 U.S.C. §119(e), of U.S. provisional applications 60/233,180, 60/279,056, and Attorney Docket 20867PV2 (serial number unassigned), filed September 15, 2000, March 27, 2001, and September 7, 2001, respectively.

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## STATEMENT REGARDING FEDERALLY-SPONSORED R&amp;D

Not Applicable

## REFERENCE TO MICROFICHE APPENDIX

15

Not Applicable

## FIELD OF THE INVENTION

The present invention relates to recombinant, replication-deficient first generation adenovirus vaccines found to exhibit enhanced growth properties and  
20 greater cellular-mediated immunity as compared to other replication-deficient vectors. The invention also relates to the associated first generation adenoviral vectors described herein, which, through the incorporation of additional 5' adenovirus sequence, enhance large scale production efficiency of the recombinant, replication-defective adenovirus described herein. Another aspect of the instant invention is the  
25 surprising discovery that the intron A portion of the human cytomegalovirus (hCMV) promoter constitutes a region of instability in adenoviral vector constructs. Removal of this region from adenoviral expression constructs results in greatly improved vector stability. Therefore, improved vectors expressing a transgene under the control of an intron A-deleted CMV promoter constitute a further aspect of this invention. These  
30 adenoviral vectors are useful for generating recombinant adenovirus vaccines against human immunodeficiency virus (HIV). In particular, the first generation adenovirus vectors disclosed herein are utilized to construct and generate adenovirus-based HIV-1 vaccines which contain HIV-1 Gag, HIV-1 Pol and/or HIV-1 Nef polynucleotide pharmaceutical products, and biologically active modifications thereof. Host  
35 administration of the recombinant, replication-deficient adenovirus vaccines described herein results in expression of HIV-1 Gag, HIV-1- Pol and/or Nef protein or

immunologically relevant modifications thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding codon optimized HIV-1 Gag, HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNase H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef, and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The HIV adenovirus vaccines of the present invention, when administered alone or in a combined modality and/or prime/boost regimen, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.

#### BACKGROUND OF THE INVENTION

Human Immunodeficiency Virus-1 (HIV-1) is the etiological agent of acquired human immune deficiency syndrome (AIDS) and related disorders. HIV-1 is an RNA virus of the Retroviridae family and exhibits the 5' LTR-*gag-pol-env*-LTR 3' organization of all retroviruses. The integrated form of HIV-1, known as the provirus, is approximately 9.8 Kb in length. Each end of the viral genome contains flanking sequences known as long terminal repeats (LTRs). The HIV genes encode at least nine proteins and are divided into three classes; the major structural proteins (Gag, Pol, and Env), the regulatory proteins (Tat and Rev); and the accessory proteins (Vpu, Vpr, Vif and Nef).

The *gag* gene encodes a 55-kilodalton (kDa) precursor protein (p55) which is expressed from the unspliced viral mRNA and is proteolytically processed by the HIV protease, a product of the *pol* gene. The mature p55 protein products are p17 (matrix), p24 (capsid), p9 (nucleocapsid) and p6.

The *pol* gene encodes proteins necessary for virus replication; a reverse transcriptase, a protease, integrase and RNase H. These viral proteins are expressed as a Gag-Pol fusion protein, a 160 kDa precursor protein which is generated via a ribosomal frame shifting. The viral encoded protease proteolytically cleaves the Pol polypeptide away from the Gag-Pol fusion and further cleaves the Pol polypeptide to the mature proteins which provide protease (Pro, P10), reverse transcriptase (RT, P50), integrase (IN, p31) and RNase H (RNase, p15) activities.

The *nef* gene encodes an early accessory HIV protein (Nef) which has been shown to possess several activities such as down regulating CD4 expression, disturbing T-cell activation and stimulating HIV infectivity.

5 The *env* gene encodes the viral envelope glycoprotein that is translated as a 160-kilodalton (kDa) precursor (gp160) and then cleaved by a cellular protease to yield the external 120-kDa envelope glycoprotein (gp120) and the transmembrane 41-kDa envelope glycoprotein (gp41). Gp120 and gp41 remain associated and are displayed on the viral particles and the surface of HIV-infected cells.

10 The *tat* gene encodes a long form and a short form of the Tat protein, a RNA binding protein which is a transcriptional transactivator essential for HIV-1 replication.

The *rev* gene encodes the 13 kDa Rev protein, a RNA binding protein. The Rev protein binds to a region of the viral RNA termed the Rev response element (RRE). The Rev protein promotes transfer of unspliced viral RNA from the nucleus  
15 to the cytoplasm. The Rev protein is required for HIV late gene expression and in turn, HIV replication.

Gp120 binds to the CD4/chemokine receptor present on the surface of helper T-lymphocytes, macrophages and other target cells in addition to other co-receptor molecules. X4 (macrophage tropic) virus show tropism for CD4/CXCR4 complexes  
20 while a R5 (T-cell line tropic) virus interacts with a CD4/CCR5 receptor complex. After gp120 binds to CD4, gp41 mediates the fusion event responsible for virus entry. The virus fuses with and enters the target cell, followed by reverse transcription of its single stranded RNA genome into the double-stranded DNA via a RNA dependent DNA polymerase. The viral DNA, known as provirus, enters the cell nucleus, where  
25 the viral DNA directs the production of new viral RNA within the nucleus, expression of early and late HIV viral proteins, and subsequently the production and cellular release of new virus particles. Recent advances in the ability to detect viral load within the host shows that the primary infection results in an extremely high generation and tissue distribution of the virus, followed by a steady state level of virus  
30 (albeit through a continual viral production and turnover during this phase), leading ultimately to another burst of virus load which leads to the onset of clinical AIDS. Productively infected cells have a half life of several days, whereas chronically or latently infected cells have a 3-week half life, followed by non-productively infected cells which have a long half life (over 100 days) but do not significantly contribute to  
35 day to day viral loads seen throughout the course of disease.

Destruction of CD4 helper T lymphocytes, which are critical to immune defense, is a major cause of the progressive immune dysfunction that is the hallmark of HIV infection. The loss of CD4 T-cells seriously impairs the body's ability to fight most invaders, but it has a particularly severe impact on the defenses against viruses, fungi, parasites and certain bacteria, including mycobacteria.

Effective treatment regimens for HIV-1 infected individuals have become available recently. However, these drugs will not have a significant impact on the disease in many parts of the world and they will have a minimal impact in halting the spread of infection within the human population. As is true of many other infectious diseases, a significant epidemiologic impact on the spread of HIV-1 infection will only occur subsequent to the development and introduction of an effective vaccine. There are a number of factors that have contributed to the lack of successful vaccine development to date. As noted above, it is now apparent that in a chronically infected person there exists constant virus production in spite of the presence of anti-HIV-1 humoral and cellular immune responses and destruction of virally infected cells. As in the case of other infectious diseases, the outcome of disease is the result of a balance between the kinetics and the magnitude of the immune response and the pathogen replicative rate and accessibility to the immune response. Pre-existing immunity may be more successful with an acute infection than an evolving immune response can be with an established infection. A second factor is the considerable genetic variability of the virus. Although anti-HIV-1 antibodies exist that can neutralize HIV-1 infectivity in cell culture, these antibodies are generally virus isolate-specific in their activity. It has proven impossible to define serological groupings of HIV-1 using traditional methods. Rather, the virus seems to define a serological "continuum" so that individual neutralizing antibody responses, at best, are effective against only a handful of viral variants. Given this latter observation, it would be useful to identify immunogens and related delivery technologies that are likely to elicit anti-HIV-1 cellular immune responses. It is known that in order to generate CTL responses antigen must be synthesized within or introduced into cells, subsequently processed into small peptides by the proteasome complex, and translocated into the endoplasmic reticulum/Golgi complex secretory pathway for eventual association with major histocompatibility complex (MHC) class I proteins. CD8<sup>+</sup> T lymphocytes recognize antigen in association with class I MHC via the T cell receptor (TCR) and the CD8 cell surface protein. Activation of naive CD8<sup>+</sup> T cells into activated effector or memory cells generally requires both TCR engagement of antigen as described above as well as engagement of costimulatory proteins. Optimal

induction of CTL responses usually requires "help" in the form of cytokines from CD4<sup>+</sup> T lymphocytes which recognize antigen associated with MHC class II molecules via TCR and CD4 engagement.

European Patent Applications 0 638 316 (Published February 15, 1995) and 0 586 076 (Published March 9, 1994), (both assigned to American Home Products Corporation) describe replicating adenovirus vectors carrying an HIV gene, including *env* or *gag*. Various treatment regimens were used with chimpanzees and dogs, some of which included booster adenovirus or protein plus alum treatments.

Replication-defective adenoviral vectors harboring deletions in the E1 region are known, and recent adenoviral vectors have incorporated the known packaging repeats into these vectors; e.g., see EP 0 707 071, disclosing, *inter alia*, an adenoviral vector deleted of E1 sequences from base pairs 459 to 3328; and U.S. Patent No. 6,033,908, disclosing, *inter alia*, an adenoviral vector deleted of base pairs 459-3510. The packaging efficiency of adenovirus has been taught to depend on the number of incorporated individual A (packaging) repeats; see, e.g., Gräble and Hearing, 1990 *J. Virol.* 64(5):2047-2056; Gräble and Hearing, 1992 *J. Virol.* 66(2):723-731.

Larder, et al., (1987, *Nature* 327: 716-717) and Larder, et al., (1989, *Proc. Natl. Acad. Sci.* 86: 4803-4807) disclose site specific mutagenesis of HIV-1 RT and the effect such changes have on *in vitro* activity and infectivity related to interaction with known inhibitors of RT.

Davies, et al. (1991, *Science* 252:, 88-95) disclose the crystal structure of the RNase H domain of HIV-1 Pol.

Schatz, et al. (1989, *FEBS Lett.* 257: 311-314) disclose that mutations Glu478Gln and His539Phe in a complete HIV-1 RT/RNase H DNA fragment results in defective RNase activity without effecting RT activity.

Mizrahi, et al. (1990, *Nucl. Acids. Res.* 18: pp. 5359-5353) disclose additional mutations Asp443Asn and Asp498Asn in the RNase region of the *pol* gene which also results in defective RNase activity. The authors note that the Asp498Asn mutant was difficult to characterize due to instability of this mutant protein.

Leavitt, et al. (1993, *J. Biol. Chem.* 268: 2113-2119) disclose several mutations, including a Asp64Val mutation, which show differing effect on HIV-1 integrase (IN) activity.

Wiskerchen, et al. (1995, *J. Virol.* 69: 376-386) disclose single and double mutants, including mutation of aspartic acid residues which effect HIV-1 IN and viral replication functions.

It would be of great import in the battle against AIDS to produce a prophylactic- and/or therapeutic-based HIV vaccine which generates a strong cellular immune response against an HIV infection. The present invention addresses and meets these needs by disclosing a class of adenovirus vaccines which, upon host administration, express codon optimized and modified versions of the HIV-1 genes, *gag*, *pol* and *nef*. These recombinant, replication-defective adenovirus vaccines may be administered to a host, such as a human, alone or as part of a combined modality regimen and/or prime-boost vaccination regimen with components of the present invention and/or a distinct viral HIV DNA vaccine, non-viral HIV DNA vaccine, HIV subunit vaccine, an HIV whole killed vaccine and/or a live attenuated HIV vaccine.

#### SUMMARY OF THE INVENTION

The present invention relates to enhanced replication-defective recombinant adenovirus vaccine vectors and associated recombinant, replication-deficient adenovirus vaccines which encode various forms of HIV-1 Gag, HIV-1 Pol, and/or HIV-1 Nef, including immunologically relevant modifications of HIV-1 Gag, HIV-1 Pol and HIV-1 Nef. The adenovirus vaccines of the present invention express HIV antigens and provide for improved cellular-mediated immune responses upon host administration. Potential vaccinees include but are not limited to primates and especially humans and non-human primates, and also include any non-human mammal of commercial or domestic veterinary importance. An effect of the improved recombinant adenovirus-based vaccines of the present invention should be a lower transmission rate to previously uninfected individuals (i.e., prophylactic applications) and/or reduction in the levels of the viral loads within an infected individual (i.e., therapeutic applications), so as to prolong the asymptomatic phase of HIV-1 infection. In particular, the present invention relates to adenoviral-based vaccines which encode various forms of codon optimized HIV-1 Gag (including but in no way limited to p55 versions of codon optimized full length (FL) Gag and tPA-Gag fusion proteins), HIV-1 Pol, HIV-1 Nef, and selected modifications of immunological relevance. The administration, intracellular delivery and expression of these adenovirus vaccines elicit a host CTL and Th response. The preferred replication-defective recombinant adenoviral vaccine vectors include but are not limited to synthetic DNA molecules which (1) encode codon optimized versions of wild type HIV-1 Gag; (2) encode codon optimized versions of HIV-1 Pol; (3) encode codon optimized versions of HIV-1 Pol fusion proteins; (4) encode codon optimized versions of modified HIV-1 Pol proteins and fusion proteins, including but not limited

to *pol* modifications involving residues within the catalytic regions responsible for RT, RNase and IN activity within the host cell; (5) encode codon optimized versions of wild type HIV-1 Nef; (6) codon optimized versions of HIV-1 Nef fusion proteins; and/or (7) codon optimized versions of HIV-1 Nef derivatives, including but not limited to *nef* modifications involving introduction of an amino-terminal leader sequence, removal of an amino-terminal myristylation site and/or introduction of dileucine motif mutations. The Nef-based fusion and modified proteins, disclosed within this specification and expressed from an adenoviral-based vector vaccine this specification, may possess altered trafficking and/or host cell function while retaining the ability to be properly presented to the host MHC I complex and in turn elicit a host CTL and Th response. Examples of HIV-1 Gag, Pol and/or Nef fusion proteins include but are not limited to fusion of a leader or signal peptide at the NH<sub>2</sub>-terminal portion of the viral antigen coding region. Such a leader peptide includes but is not limited to a tPA leader peptide.

The adenoviral vector utilized in construction of the HIV-1 Gag-, HIV-1 Pol- and/or HIV-1 Nef- based vaccines of the present invention may comprise any replication-defective adenoviral vector which provides for enhanced genetic stability of the recombinant adenoviral genome through large scale production and purification of the recombinant virus. In other words, an HIV-1 Gag-, Pol- or Nef-based adenovirus vaccine of the present invention is a purified recombinant, replication-defective adenovirus which is shown to be genetically stable through multiple passages in cell culture and remains so during large scale production and purification procedures. Such a recombinant adenovirus vector and harvested adenovirus vaccine lends itself to large scale dose filling and subsequent worldwide distribution procedures which will be demanded of an efficacious monovalent or multivalent HIV vaccine. The present invention meets this basic requirement with description of a replication-defective adenoviral vector and vectors derived therefrom, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 of the wildtype adenovirus genome. A preferred embodiment of the instant invention comprises base pairs 1-450 of a wildtype adenovirus. In other preferred embodiments, the replication -defective adenoviral vector has, in addition thereto, a region 3' to the E1-deleted region comprising base pairs 3511-3523. Basepairs 342-450 (more particularly, 400-450) constitute an extension of the 5' region of previously disclosed vectors carrying viral antigens, particularly HIV antigens (see, e.g., PCT International Application PCT/US00/18332, published

January 11, 2001 (WO 01/02067), which claims priority to U.S. Provisional Application Serial Nos. 60/142,631 and 60/148,981, filed 7/6/1999 and 8/13/1999, respectively; these documents herein incorporated by reference. Applicants have found that extending the 5' region further into the E1 gene into the disclosed vaccine  
5 vectors incorporated elements found to be important in optimizing the packaging of the virus.

As compared to previous vectors not comprising basepairs from about 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 of the wildtype adenovirus genome, vectors comprising the above region exhibited enhanced  
10 growth characteristics, with approximately 5-10 fold greater amplification rates, a more potent virus effect, allowing lower doses of virus to be used to generate equivalent immunity; and a greater cellular-mediated immune response than replication-deficient vectors not comprising this region (basepairs 1-450). Even more important, adenoviral constructs derived therefrom are very stable genetically in  
15 large-scale production, particularly those comprising an expression cassette under the control of a hCMV promoter devoid of intron A. This is because Applicants have surprisingly found that the intron A portion of the hCMV promoter constituted a region of instability when employed in adenoviral vectors. Applicants have, therefore, identified an enhanced adenoviral vector which is particularly suited for use  
20 in gene therapy and nucleotide-based vaccine-vectors which, favorably, lends itself to large scale propagation.

A preferred embodiment of this invention is a replication-defective adenoviral vector in accordance with the above description wherein the gene is inserted in the form of a gene expression cassette comprising (a) a nucleic acid encoding a protein or  
25 biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and, (c) a transcription terminator.

In preferred embodiments, the E1 gene, other than that contained within basepairs 1-450 or, alternatively, that contained within base pairs 1-450 and 3511-  
30 3523 has been deleted from the adenoviral vector, and the gene expression cassette has replaced the deleted E1 gene. In other preferred embodiments, the replication defective adenovirus genome does not have a functional E3 gene, or the E3 gene has been deleted. Most preferably, the E3 region is present within the adenoviral genome. Further preferred embodiments are wherein the gene expression cassette is in an E1  
35 anti-parallel (transcribed in a 3' to 5' direction relative to the vector backbone)



orientation or, more preferably, an E1 parallel (transcribed in a 5' to 3' direction relative to the vector backbone) orientation.

Further embodiments relate to a shuttle plasmid vector comprising: an adenoviral portion and a plasmid portion, wherein said adenovirus portion comprises:

- 5 a) a replication defective adenovirus genome, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 (preferably, 1-450) of the wildtype adenovirus genome and, preferably, in addition thereto, basepairs 3511-3523 of a wildtype adenovirus sequence; and b) a gene  
10 expression cassette comprising: (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and (c) a transcription terminator and/or a polyadenylation site.

- Other aspects of this invention include a host cell comprising said adenoviral  
15 vectors and/or said shuttle plasmid vectors; vaccine compositions comprising said vectors; and methods of producing the vectors comprising (a) introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and (b) harvesting the resultant adenoviral vectors.

- To this end, the present invention particularly relates to harvested  
20 recombinant, replication defective virus derived from a host cell, such as but not limited to 293 cells or PER.C6<sup>®</sup> cells, including but not limited to harvested virus related to any of the MRKAd5 vector backbones, with or without an accompanying transgene, including but not limited to the HIV-1 antigens described herein. An HIV-1 vaccine is represented by any harvested, recombinant adenovirus material  
25 which expresses any one or more of the HIV-1 antigens disclosed herein. This harvested material may then be purified, formulated and stored prior to host administration.

- Another aspect of this invention is a method of generating a cellular immune response against a protein in an individual comprising administering to the individual  
30 an adenovirus vaccine vector comprising:

- a) a recombinant, replication defective adenoviral vector, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting adenovirus packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 (preferably, 1-450) and, preferably in addition thereto,  
35 base pairs 3511-3523 of a wildtype adenovirus sequence, and,

b) a gene expression cassette comprising: (i) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (ii) a heterologous promoter operatively linked to the nucleic acid of part a); and (iii) a transcription terminator and/or a polyadenylation site.

5 In view of the efficacious nature of the adenoviral and/or DNA plasmid vaccines described herein, the present invention relates to all methodology regarding administration of one or more of these adenoviral and/or DNA plasmid vaccines to provide effective immunoprophylaxis, to prevent establishment of an HIV-1 infection following exposure to this virus, or as a post-HIV infection therapeutic vaccine to  
10 mitigate the acute HIV-1 infection so as to result in the establishment of a lower virus load with beneficial long term consequences. As discussed herein, such a treatment regimen may include a monovalent or multivalent composition, various combined modality applications, and/or a prime/boost regimen to as to optimize antigen expression and a concomitant cellular-mediated and/or humoral immune response  
15 upon inoculation into a living vertebrate tissue. Therefore, the present invention provides for methods of using the adenoviral and/or DNA plasmid vaccines disclosed herein within the various parameters disclosed herein as well as any additional parameters known in the art, which, upon introduction into mammalian tissue induces intracellular expression of the gag, pol and/or nef-based vaccines.

20 To this end, the present invention relates in part to methods of generating a cellular immune response in a vaccinee, preferably a human vaccinee, wherein the individual is given more than one administration of adenovirus vaccine vector, and it may be given in a regimen accompanied by the administration of a plasmid vaccine. The plasmid vaccine (also referred to herein as a "DNA plasmid vaccine" or "vaccine  
25 plasmid" comprises a nucleic acid encoding a protein or an immunologically relevant portion thereof, a heterologous promoter operably linked to the nucleic acid sequence, and a transcription terminator or a polyadenylation signal (such as bGH or SPA, respectively). There may be a predetermined minimum amount of time separating the administrations. The individual can be given a first dose of plasmid vaccine, and then  
30 a second dose of plasmid vaccine. Alternatively, the individual may be given a first dose of adenovirus vaccine, and then a second dose of adenovirus vaccine. In other embodiments, the plasmid vaccine is administered first, followed after a time by administration of the adenovirus vaccine. Conversely, the adenovirus vaccine may be administered first, followed by administration of plasmid vaccine after a time. In  
35 these embodiments, an individual may be given multiple doses of the same adenovirus serotype in either viral vector or plasmid form, or the virus may be of

differing serotypes. In the alternative, a viral antigen of interest can be first delivered via a viral vaccine other than an adenovirus-based vaccine, and then followed with the adenoviral vaccine disclosed. Alternative viral vaccines include but are not limited to pox virus and venezuelan equine encephalitis virus.

5           The present invention also relates to multivalent adenovirus vaccine compositions which comprise Gag, Pol and Nef components described herein; see, e.g., Example 29 and Table 25. Such compositions will provide for an enhanced cellular immune response subsequent to host administration, particularly given the genetic diversity of human MHCs and of circulating virus. Examples, but not  
10       limitations, include MRKAd5-vector based multivalent vaccine compositions which provide for a divalent (i.e., gag and nef, gag and pol, or pol and nef components) or a trivalent vaccine (i.e., gag, pol and nef components) composition. Such a multivalent vaccine may be filled for a single dose or may consist of multiple inoculations of each individually filled component; and may in addition be part of a prime/boost regimen  
15       with viral or non-viral vector vaccines as introduced in the previous paragraph. To this end, preferred compositions are MRKAd5 adenovirus used in combination with multiple, distinct HIV antigen classes. Each HIV antigen class is subject to sequence manipulation, thus providing for a multitude of potential vaccine combinations; and such combinations are within the scope of the present invention. The utilization of  
20       such combined modalities vaccine formulation and administration increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

          The concept of a "combined modality" as disclosed herein also covers the alternative mode of administration whereby multiple HIV-1 viral antigens may be  
25       ligated into a proper shuttle plasmid for generation of a pre-adenoviral plasmid comprising multiple open reading frames. For example, a trivalent vector may comprise a gag-pol-nef fusion, in either a E3(-) or E3(+) background, preferably a E3 deleted backbone, or possibly a "2+1" divalent vaccine, such as a gag-pol fusion (i.e., codon optimized p55 gag and inactivated optimized pol; Example 29 and Table 25)  
30       within the same MRKAd5 backbone, with each open reading frame being operatively linked to a distinct promoter and transcription termination sequence. Alternatively, the two open reading frames may be operatively linked to a single promoter, with the open reading frames operatively linked by an internal ribosome entry sequence (IRES). Therefore, a multivalent vaccine delivered as a single, or possibly a second  
35       harvested recombinant, replication-deficient adenovirus is contemplated as part of the present invention.

Therefore, the adenoviral vaccines and plasmid DNA vaccines of this invention may be administered alone, or may be part of a prime and boost administration regimen. A mixed modality priming and booster inoculation scheme will result in an enhanced immune response, particularly if pre-existing anti-vector immune responses are present. This one aspect of this invention is a method of priming a subject with the plasmid vaccine by administering the plasmid vaccine at least one time, allowing a predetermined length of time to pass, and then boosting by administering the adenoviral vaccine. Multiple primings typically, 1-4, are usually employed, although more may be used. The length of time between priming and boost may typically vary from about four months to a year, but other time frames may be used. In experiments with rhesus monkeys, the animals were primed four times with plasmid vaccines, then were boosted 4 months later with the adenoviral vaccine. Their cellular immune response was notably higher than that of animals which had only received adenoviral vaccine. The use of a priming regimen may be particularly preferred in situations where a person has a pre-existing anti-adenovirus immune response.

It is an object of the present invention to provide for enhanced replication-defective recombinant adenoviral vaccine vector backbones. These recombinant adenoviral backbones may accept one or more transgenes, which may be passaged through cell culture for growth, amplification and harvest.

It is a further object to provide for enhanced replication-defective recombinant adenoviral vaccine vectors which encode various transgenes.

It is also an object of the present invention to provide for a harvested recombinant, replication-deficient adenovirus which shows enhanced growth and amplification rates while in combination with increased virus stability after continuous passage in cell culture. Such a recombinant adenovirus is particularly suited for use in gene therapy and nucleotide-based vaccine vectors which, favorably, lends itself to large scale propagation.

To this end, it is an object of the present invention to provide for (1) enhanced replication-defective recombinant adenoviral vaccine vectors as described herein which encode various forms of HIV-1 Gag, HIV-1 Pol, and/or HIV-1 Nef, including immunologically relevant modifications of HIV-1 Gag, HIV-1 Pol and HIV-1 Nef, and (2) harvested, purified recombinant replication-deficient adenovirus generated by passage of the adenoviral vectors of (1) through one or multiple passages through cell culture, including but not limited to passage through 293 cells or PER.C6® cells.

It is also an object of the present invention to provide for recombinant adenovirus harvested by one or multiple passages through cell culture. As relating to recombinant adenoviral vaccine vector, this recombinant virus is harvested and formulated for subsequent host administration.

5 It is also an object of the present invention to provide for replication-defective adenoviral vectors wherein at least one gene is inserted in the form of a gene expression cassette comprising (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and, (c) a transcription terminator.

10 It is also an object of the present invention to provide for a host cell comprising said adenoviral vectors and/or said shuttle plasmid vectors; vaccine compositions comprising said vectors; and methods of producing the vectors comprising (a) introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and (b) harvesting the resultant adenoviral vectors.

15 It is a further object of the present invention to provide for methods of generating a cellular immune response against a protein in an individual comprising administering to the individual an adenovirus vaccine vector comprising a) a replication defective adenoviral vector, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting packaging region from about base pair 1 to between from about base pair 20 342 (more preferably, 400) to about 450 (preferably, 1-450) and, preferably, 3511-3523 of a wildtype adenovirus sequence, and, b) a gene expression cassette comprising: (i) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (ii) a heterologous promoter operatively linked to the nucleic acid of part a); and (iii) a transcription terminator and/or a 25 polyadenylation site.

It is also an object of the present invention to provide various alternatives for vaccine administration regimes, namely administration of one or more adenoviral and/or DNA plasmid vaccines described herein to provide effective immunoprophylaxis for uninfected individuals or a therapeutic treatment for HIV 30 infected patients. Such processes include but are not limited to multivalent HIV-1 vaccine compositions, various combined modality regimes as well as various prime/boost alternatives. These methods of administration, relating to vaccine composition and/or scheduled administration, will increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a 35 single modality regimen.

As used throughout the specification and claims, the following definitions and abbreviations are used:

"HAART" refers to -- highly active antiretroviral therapy --.

"first generation" vectors are characterized as being replication-defective.

5 They typically have a deleted or inactivated E1 gene region, and preferably have a deleted or inactivated E3 gene region as well.

"AEX" refers to Anion Exchange chromatography.

"QPA" refers to Quick PCR-based Potency Assay.

"bps" refers to basepairs.

10 "s" or "str" denotes that the transgene is in the E1 parallel or "straight" orientation.

"PBMCs" refers to peripheral blood monocyte cells.

"FL" refers to full length.

"FLgag" refers to a full-length optimized gag gene, as shown in Figure 2.

15 "Ad5-Flag" refers to an adenovirus serotype 5 replication deficient virus which carries an expression cassette which comprises a full length optimized gag gene under the control of a CMV promoter.

"Promoter" means a recognition site on a DNA strand to which an RNA polymerase binds. The promoter forms an initiation complex with RNA polymerase  
20 to initiate and drive transcriptional activity. The complex can be modified by activating sequences such as enhancers or inhibiting sequences such as silencers.

"Leader" means a DNA sequence at the 5' end of a structural gene which is transcribed along with the gene. This usually results a protein having an N-terminal peptide extension, often referred to as a pro-sequences.

25 "Intron" means a section of DNA occurring in the middle of a gene which does not code for an amino acid in the gene product. The precursor RNA of the intron is excised and is therefore not transcribed into mRNA not translated into protein.

"Immunologically relevant" or "biologically active" means (1) with regards to a viral protein, that the protein is capable, upon administration, of eliciting a  
30 measurable immune response within an individual sufficient to retard the propagation and/or spread of the virus and/or to reduce the viral load present within the individual; or (2) with regards to a nucleotide sequence, that the sequence is capable of encoding for a protein capable of the above.

"Cassette" refers to a nucleic acid sequence which is to be expressed, along  
35 with its transcription and translational control sequences. By changing the cassette, a vector can express a different sequence.

"bGHpA" refers to the bovine growth hormone transcription terminator/polyadenylation sequence.

"tPAgag" refers to a fusion between the leader sequence of the tissue plasminogen activator leader sequence and an optimized HIV gag gene, as exemplified in Figure 30A-B, whether in a DNA or adenovirus-based vaccine vector.

Where utilized, "IA" or "inact" refers to an inactivated version of a gene (e.g. IApol).

"MCS" is "multiple cloning site".

In general, adenoviral constructs, gene constructs are named by reference to the genes contained therein. For example:

"Ad5 HIV-1 gag", also referred to as the original HIV-1 gag adenoviral vector, is a vector containing a transgene cassette composed of a hCMV intron A promoter, the full length version of the human codon-optimized HIV-1 gag gene, and the bovine growth hormone polyadenylation signal. The transgene was inserted in the E1 antiparallel orientation in an E1 and E3 deleted adenovector.

"MRK Ad5 HIV-1 gag" also referred to as "MRKAd5gag" or "Ad5gag2" is an adenoviral vector taught herein which is deleted of E1, comprises basepairs 1-450 and 3511-3523, and has a human codon-optimized HIV-1 gene in an E1 parallel orientation under the control of a CMV promoter without intron A. The construct also comprises a bovine growth hormone polyadenylation signal.

"pV1JnsHIVgag", also referred to as "HIVFLgagPR9901", is a plasmid comprising the CMV immediate-early (IE) promoter and intron A, a full-length codon-optimized HIV gag gene, a bovine growth hormone-derived polyadenylation and transcriptional termination sequence, and a minimal pUC backbone.

"pV1JnsCMV(no intron)-FLgag-bGHpA" is a plasmid derived from pV1JnsHIVgag which is deleted of the intron A portion of CMV and which comprises the full length HIV gag gene. This plasmid is also referred to as "pV1JnsHIVgag-bGHpA", pV1Jns-hCMV-FL-gag-bGHpA" and "pV1JnsCMV(no intron) + FLgag + bGHpA".

"pV1JnsCMV(no intron)-FLgag-SPA" is a plasmid of the same composition as pV1JnsCMV(no intron)-FLgag-bGHpA except that the SPA termination sequence replaces that of bGHpA. This plasmid is also referred to as "pV1Jns-HIVgag-SPA" and pV1Jns-hCMV-FLgag-SPA".

"pdelE1sp1A" is a universal shuttle vector with no expression cassette (i.e., no promoter or polyA). The vector comprises wildtype adenovirus serotype 5 (Ad5) sequences from bp 1 to bp 341 and bp 3524 to bp 5798, and has a multiple cloning

site between the Ad5 sequences ending 341 bp and beginning 3524 bp. This plasmid is also referred to as the original Ad 5 shuttle vector.

"MRKpdelE1sp1A" or "MRKpdelE1(Pac/pIX/pack450)" or

5 "MRKpdelE1(Pac/pIX/pack450)Cla1" is a universal shuttle vector with no expression cassette (i.e. no promoter or polyA) comprising wildtype adenovirus serotype 5 (Ad5) sequences from bp1 to bp450 and bp 3511 to bp 5798. The vector has a multiple cloning site between the Ad5 sequence ending 450 bp and beginning 3511 bp. This shuttle vector may be used to insert the CMV promoter and the bGHpA fragments in both the straight ("str". or E1 parallel) orientation or in the opposite (opp. or E1  
10 antiparallel) orientation)

"MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.)" is still another shuttle vector which is the modified vector that contains the CMV promoter (no intronA) and the bGHpA fragments. The expression unit containing the hCMV promoter (no intron A) and the bovine growth hormone polyadenylation signal has  
15 been inserted into the shuttle vector such that insertion of the gene of choice at a unique *Bgl*III site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1/E3+)Cla1 pre-plasmid. This shuttle vector, as shown in Figures 22 and 23, was used to insert the respective IAPol and G2A,LLAA nef genes directly into.

20 "MRKpdelE1-CMV(no intron)-FLgag-bGHpA" is a shuttle comprising Ad5 sequences from basepairs 1-450 and 3511-5798, with an expression cassette containing human CMV without intron A, the full-length human codon-optimized HIV gag gene and bovine growth hormone polyadenylation signal. This plasmid is also referred to as "MRKpdelE1 shuttle +hCMV-FL-gag-BGHpA"

25 "MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA" is an adenoviral vector comprising all Ad5 sequences except those nucleotides encompassing the E1 region (from 451-3510), a human CMV promoter without intron A, a full-length human codon-optimized HIV gag gene, and a bovine growth hormone polyadenylation signal. This vector is also referred to as "MRKpAdHVE3 + hCMV-FL-gag-  
30 BGHpA", "MRKpAd5HIV-1gag", "MRKpAd5gag", "pMRKAd5gag" or "pAd5gag2".

"pV1Jns-HIV-pol inact(opt)" or "pV1Jns-HIV IA pol (opt)" is the inactivated Pol gene (contained within SEQ ID NO:3) cloned into the *Bgl*III site of V1Jns (Figure 17A-C). As noted herein, various derivatives of HIV-1 pol may be cloned into a  
35 plasmid expression vector such as V1Jns or V1Jns-tPA, thus serving directly as DNA vaccine candidates or as a source for subcloning into an appropriate adenoviral vector.



"MRKpdel+hCMVmin+FL-pol+bGHpA(s)" is the "MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHPA(str.)" shuttle mentioned above which contains the IA pol gene in the proper orientation. This shuttle vector is used in a bacterial recombination with MRKpAd(E1-/E3+)Cla1.

5 "MRKpAd+hCMVmin+FL-pol+bGHpA(S)E3+", also referred to herein as "pMRKAd5pol", is the pre-adenovirus plasmid which comprises a CMV-pol inact(opt)-pGHpA construct. The construction of this pre-adenovirus plasmid is shown in Figure 22.

10 "pV1Jns/nef (G2A,LLAA)" or "V1Jns/opt nef (G2A,LLAA)" comprises codon optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175 (SEQ ID NO:13; which comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662). This fragment is subcloned into the Bgl II site of V1Jns and/or V1Jns-tPA (Figures 16A-B). As noted above for HIV-1 pol, HIV-1 nef constructs may be cloned into a plasmid expression vector such as V1Jns or V1Jns-tPA, thus serving directly as DNA vaccine candidates or as a source for subcloning into an appropriate adenoviral vector.

15 "MRKpdelE1hCMVminFL-nefBGHPA(s)", also referred to herein as "pMRKAd5nef", is the pre-adenovirus plasmid which comprises a CMV-nef (G2A,LLAA) codon optimized sequence. The construction of this pre-adenovirus plasmid is shown in Figure 23.

#### BRIEF DESCRIPTION OF THE FIGURES

25 Figure 1 shows the original HIV-1 gag adenovector (Ad5HIV-1gag). This vector is disclosed in PCT International Application No. PCT/US00/18332 (WO 01/02607) filed July 3, 2000, claiming priority to U.S. Provisional Application Serial No. 60/142,631, filed July 6, 1999 and U.S. Application Serial No. 60/148,981, filed August 13, 1999, all three applications which are hereby incorporated by reference.

30 Figure 2 shows the nucleic acid sequence (SEQ ID NO: 29) of the optimized human HIV-1 gag open reading frame.

Figure 3 shows diagrammatically the new transgene constructs in comparison with the original gag transgene.

35 Figure 4 shows the modifications made to the original adenovector backbone in the generation of the novel vectors of the instant invention.

Figure 5 shows the virus mixing experiments that were carried out to determine the effects of the addition made to the packaging signal region (Expt. #1) and the E3 gene on viral growth (Expt. #2). The bars denote the region of modifications made to the E1 deletion:

5        Figure 6 shows an autoradiograph of viral DNA analysis following the viral mixing experiments described in Examples 6 and 7.

Figures 7A, 7B and 7C are as follows: Figure 7A shows the hCMV-Flagg-bGHpA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Both E1 parallel and E1 antiparallel transgene orientation are represented. Figure 7B shows the hCMV-Flagg-SPA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Again, both E1 parallel and E1 antiparallel transgene orientation are represented. Figure 7C shows the mCMV-Flagg-bGHpA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Once again, both E1 parallel and E1 antiparallel transgene orientation are represented.

Figure 8A shows the experiment designed to test the effect of transgene orientation.

Figure 8B shows the experiments designed to test the effect of polyadenylation signal.

20        Figure 9 shows viral DNA from the four adenoviral vectors tested (Example 12) at P5, following *Bst*E11 digestion.

Figure 10 shows viral DNA analysis of passages 11 and 12 of MRKpAdHVE3, MRKAd5HIV-1gag, and MRKAd5HIV-1gagE3-.

Figure 11 shows viral DNA analysis (*Hind*III digestion) of passage 6 MRKpAdHVE3 and MRKAd5HIV-1gag used to initiate the viral competition study. The last two lanes are passage 11 analysis of duplicate passages of the competition study (each virus at MOI of 280 viral particles).

Figure 12 shows viral DNA analysis by *Hind* III digestion on high passage numbers for MRKAd5HIV-1gag in serum-containing media with collections made at specified times. The first lane shows the 1kb DNA size marker. The other lanes represent pre-plasmid control (digested with *Pac*I and *Hind*III), MRKAd5HIV-1gag at P16, P19, and P21.

Figure 13 shows serum anti-p24 levels at 3 wks post i.m. immunization of balb/c mice (n=10) with varying doses of several Adgag constructs: (A) MRK Ad5 HIV-1 gag (through passage 5); (B) MRKAd5 hCMV-FLgag-bGHpA (E3-); (C) MRKAd5 hCMV-FLgag-SPA (E3+); (D) MRKAd5 mCMV-FLgag-bGHpA (E3+);

(E) research lot (293 cell-derived) of Ad5HIV-1 gag; and (F) clinical lot (Ad5gagFN0001) of Ad5HIV-1 gag. Reported are the geometric mean titers (GMT) for each cohort along with the standard error bars.

Figure 14 shows a restriction map of the pMRKAd5HIV-1gag vector.

5        Figures 15A-X illustrates the nucleotide sequence of the pMRKAd5HIV-1gag vector (SEQ ID NO:27.[coding] and SEQ ID NO:28 [non-coding]).

Figures 16A-B shows a schematic representation of DNA vaccine expression vectors V1Jns (A) and V1Jns-tPA (B), which are utilized for HIV-1 gag, pol and nef constructs in various DNA/viral vector combined modality regimens as disclosed  
10        herein.

Figures 17A-C shows the nucleotide (SEQ ID NO:3) and amino acid sequence (SEQ ID NO:4) of IA-Pol. Underlined codons and amino acids denote mutations, as listed in Table 1.

Figure 18 shows codon optimized nucleotide and amino acid sequences  
15        through the fusion junction of tPA-pol inact(opt) (contained within SEQ ID NOs: 7 and 8, respectively). The underlined portion represents the NH<sub>2</sub>-terminal region of IA-Pol.

Figures 19A-B show a nucleotide sequence comparison between wild type nef(jrfl) and codon optimized nef. The wild type nef gene from the jrfl isolate  
20        consists of 648 nucleotides capable of encoding a 216 amino acid polypeptide. WT, wild type sequence (SEQ ID NO:19); opt, codon-optimized sequence (contained within SEQ ID NO:1). The Nef amino acid sequence is shown in one-letter code (SEQ ID NO:2).

Figures 20A-C show nucleotide sequences at junctions between nef coding  
25        sequence and plasmid backbone of nef expression vectors V1Jns/nef (Figure 20A), V1Jns/nef(G2A,LLAA) (Figure 20B), V1Jns/tpanef (Figure 20C) and V1Jns/tpanef(LLAA) (Figure 20C, also). 5' and 3' flanking sequences of codon optimized nef or codon optimized nef mutant genes are indicated by bold/italic letters; nef and nef mutant coding sequences are indicated by plain letters. Also indicated (as  
30        underlined) are the restriction endonuclease sites involved in construction of respective nef expression vectors. V1Jns/tpanef and V1Jns/tpanef(LLAA) have identical sequences at the junctions.

Figure 21 shows a schematic presentation of nef and nef derivatives. Amino  
acid residues involved in Nef derivatives are presented. Glycine 2 and Leucine174  
35        and 175 are the sites involved in myristylation and dileucine motif, respectively. For both versions of the tpanef fusion genes, the putative leader peptide cleavage sites are

indicated with "\*", and a exogenous serine residue introduced during the construction of the mutants is underlined.

Figure 22 shows diagrammatically the construction of the pre-adenovirus plasmid construct, MRKAd5Pol.

5        Figure 23 shows diagrammatically the construction of the pre-adenovirus plasmid construct, MRKAd5Nef.

Figure 24 shows a comparison of clade B vs. clade C anti-gag T cell responses in clade B HIV-infected subjects.

10       Figure 25 shows a comparison of clade B vs. clade C anti-nef T cell responses in clade B HIV-infected subjects.

Figures 26A-AO illustrates the nucleotide sequence of the pMRKAd5HIV-1pol adenoviral vector (SEQ ID NO:32 [coding] and SEQ ID NO:33 [non-coding]), comprising the coding region of the inactivated pol gene (SEQ ID NO3).

15       Figures 27A-AM illustrates the nucleotide sequence of the pMRKAd5HIV-1 nef adenoviral vector (SEQ ID NO:34 [coding] and SEQ ID NO:35 [non-coding]), comprising the coding region of the inactivated pol gene (SEQ ID NO13).

Figure 28 shows the stability of MRKAd5 vectors comprising various promoter fragments (hCMV or mCMV) and terminations signals (bGH or SPA) in E3(+) or E3(-) backbones.

20       Figures 29A and B shows the anion-exchange HPLC viral particle concentrations of the freeze-thaw recovered cell associated virus at the 24, 36, 48, and 60 hpi time points (Figure 29A) and the timcourse QPA supernatant titers (Figure 29B) for MRKAd5gag, MRKAd5pol and MRKAd5nef.

25       Figure 30 shows the nucleotide sequence (SEQ ID NO:36) and amino acid sequence (SEQ ID NO:37) comprising the open reading frame of a representative tPA-gag fusion for use in the DNA and/or adenoviral vaccine disclosed herein.

30       Figure 31 shows the intracellular  $\gamma$ IFN staining of PBMCs collected at week 10 (post DNA prime) and week 30 (post Ad boost). The cells were stimulated overnight in the presence or absence of the gag peptide pool. They were subsequently stained using fluorescence-tagged anti-CD3, anti-CD8, anti-CD4, and anti- $\gamma$ IFN monoclonal antibodies. Each plot shows all CD3+ T cells which were segregated in terms of positive staining for surface CD8 and  $\gamma$ IFN production. The numbers in the upper right and lower right quadrants of each plot are the percentages of CD3+ cells that were CD8+ $\gamma$ IFN+ and CD4+ $\gamma$ IFN+, respectively.

35       Figure 32 shows a comparison of single-modality adenovirus immunization with DNA + adjuvant prime/adenovirus boost immunization.

Figures 33A-B show the nucleotide sequence (SEQ ID NO: 38) of the open reading frame for the gag-IAPol fusion of Example 29.

Figures 34A-B show the protein sequence (SEQ ID NO:39) of the gag-IAPol fusion frame.

5

#### DETAILED DESCRIPTION OF THE INVENTION

A novel replication-defective, or "first generation," adenoviral vector suitable for use in gene therapy or nucleotide-based vaccine vectors is described. This vector is at least partially deleted in E1 and comprises a wildtype adenovirus *cis*-acting packaging region from about base pair 1 to between about base pair 342 (more preferably, 400) to about 458 (preferably, 1-450) and, preferably, 3511-3523 of a wild-type adenovirus sequence. It has been found that a vector of this description possesses enhanced growth characteristics, with approximately 5-10 fold greater amplification rates, and is more potent allowing lower doses of virus to be used to generate equivalent immunity. The vector, furthermore, generates a harvested recombinant adenovirus which shows greater cellular-mediated immune responses than replication-deficient vectors not comprising this region (basepairs 342-450). Adenoviral constructs derived from these vectors are, further, very stable genetically, particularly those comprising a transgene under the control of a hCMV promoter devoid of intron A. Viruses in accordance with this description were passaged continually and analyzed; see Example 12. Each virus analyzed maintained its correct genetic structure. Analysis was also carried out under propagation conditions similar to that performed in large scale production. Again, the vectors were found to possess enhanced genetic stability; see Figure 12. Following 21 passages, the viral DNA showed no evidence of rearrangement, and was highly reproducible from one production lot to the next. The outcome of all relevant tests indicate that the adenoviral vector is extremely well suited for large-scale production of recombinant, replication-deficient adenovirus, as shown herein with the data associated with Figure 28.

A preferred adenoviral vector in accordance with this description is a vector comprising basepairs 1-450, which is deleted in E3. This vector can accommodate up to approximately 7,500 base pairs of foreign DNA inserts (or exogenous genetic material). Another preferred vector is one retaining E3 which comprises basepairs 1-450. A preferred vector of this description is an E3+ vector comprising basepairs 1-450 and 3511-3523. This vector, when deleted of the region spanning basepairs 451-3510, can accommodate up to approximately, 4,850 base pairs of foreign DNA inserts

(or exogenous genetic material). The cloning capacities of the above vectors have been determined using 105% of the wildtype Ad5 sequence as the upper genome size limit.

Wildtype adenovirus serotype 5 is used as the basis for the specific basepair numbers provided throughout the specification. The wildtype adenovirus serotype 5 sequence is known and described in the art; *see*, Chroboczek *et al.*, 1992 *J. Virology* 186:280, which is hereby incorporated by reference. Accordingly, a particular embodiment of the instant invention is a vector based on the adenovirus serotype 5 sequence. One of skill in the art can readily identify the above regions in other adenovirus serotypes (e.g., serotypes 2, 4, 6, 12, 16, 17, 24, 31, 33, and 42), regions defined by basepairs corresponding to the above basepair positions given for adenovirus serotype 5. Accordingly, the instant invention encompasses all adenoviral vectors partially deleted in E1 comprising basepairs corresponding to 1-450 (particularly, 342-450) and, preferably, 3511-3523 of a wild-type adenovirus serotype 5 (Ad5) nucleic acid sequence. Particularly preferred embodiments of the instant invention are those derived from adenoviruses like Ad5 which are classified in subgroup C (e.g., Ad2).

Vectors in accordance with the instant invention are at least partially deleted in E1. Preferably the E1 region is completely deleted or inactivated. Most preferably, the region deleted of E1 is within basepairs 451-3510. It is to be noted that the extended 5' and 3' regions of the disclosed vectors are believed to effectively reduce the size of the E1 deletion of previous constructs without overlapping any part of the E1A/E1B gene present in the cell line used, i.e., the PER.C6<sup>®</sup> cell line transfected with base pairs 459-3510. Overlap of adenoviral sequences is avoided because of the possibility of recombination. One of ordinary skill in the art can certainly appreciate that the instant invention can, therefore, be modified if a different cell line transfected with a different segment of adenovirus DNA is utilized. For purposes of exemplification, a 5' region of base pairs 1 to up to 449 is more appropriate if a cell line is transfected with adenoviral sequence from base pairs 450-3510. This holds true as well in the consideration of segments 3' to the E1 deletion.

Preferred embodiments of the instant invention possess an intact E3 region (i.e., an E3 gene capable of encoding a functional E3). Alternate embodiments have a partially deleted E3, an inactivated E3 region, or a sequence completely deleted of E3. Applicants have found, in accordance with the instant invention, that virus comprising the E3 gene were able to amplify more rapidly compared with virus not comprising an E3 gene; see Figure 6 wherein a diagnostic CsCl band corresponding to the E3+ virus

tested (5,665 bp) was present in greater amount compared with the diagnostic band of 3,010 bp corresponding to the E3- virus. These results were obtained following a virus competition study involving mixing equal MOI ratio (1:1) of adenovectors both comprising the E3 gene and not comprising the E3 gene. This increased amplification capacity of the E3+ adenovectors was subsequently confirmed with growth studies; see Table 4A, wherein the E3+ virus exhibit amplification ratios of 470, 420 and 320 as compared with the 115 and 40-50 of the E3- constructs.

As stated above, vectors in accordance with the instant invention can accommodate up to approximately 4,850 base pairs of exogenous genetic material for an E3+ vector and approximately 7,500 base pairs for an E3- vector. Preferably, the insert brings the adenoviral vector as close as possible to a wild-type genomic size (e.g., for Ad5, 35,935 basepairs). It is well known that adenovirus amplifies best when they are close to their wild-type genomic size.

The genetic material can be inserted in an E1-parallel or an E1 anti-parallel orientation, as such is illustrated in Figure 7A, 7B, 7C and Figure 8A. Particularly preferred embodiments of the instant invention, have the insert in an E1-parallel orientation. Applicants have found, via competition experiments with plasmids containing transgenes in differing orientation (Figure 8A), that vector constructs with the foreign DNA insert in an E1-parallel orientation amplify better and actually out-compete E1-antiparallel-oriented transgenes. Viral DNA analysis of the mixtures at passage 3 and certainly at passage 6, showed a greater ratio of the virus carrying the transgene in the E1 parallel orientation as compared with the E1 anti-parallel version. By passage 10, the only viral species observed was the adenovector with the transgene in the E1 parallel orientation for both transgenes tested.

Adenoviral vectors in accordance with the instant invention are particularly well suited to effectuate expression of desired proteins, one example of which is an HIV protein, particularly an HIV full length gag protein. Exogenous genetic material encoding a protein of interest can exist in the form of an expression cassette. A gene expression cassette preferably comprises (a) a nucleic acid encoding a protein of interest; (b) a heterologous promoter operatively linked to the nucleic acid encoding the protein; and (c) a transcription terminator.

The transcriptional promoter is preferably recognized by an eukaryotic RNA polymerase. In a preferred embodiment, the promoter is a "strong" or "efficient" promoter. An example of a strong promoter is the immediate early human cytomegalovirus promoter (Chapman et al, 1991 *Nucl. Acids Res*19:3979-3986, which is incorporated by reference), preferably without intronic sequences. Most preferred

for use within the instant adenoviral vector is a human CMV promoter without intronic sequences, like intron A. Applicants have found that intron A, a portion of the human cytomegalovirus promoter (hCMV), constitutes a region of instability for adenoviral vectors. CMV without intron A has been found to effectuate (Examples 1-3) comparable expression capabilities *in vitro* when driving HIV gag expression and, furthermore, behaved equivalently to intron A-containing constructs in Balb/c mice *in vivo* with respect to their antibody and T-cell responses at both dosages of plasmid DNA tested (20 µg and 200 µg). Those skilled in the art will appreciate that any of a number of other known promoters, such as the strong immunoglobulin, or other eukaryotic gene promoters may also be used, including the EF1 alpha promoter, the murine CMV promoter, Rous sarcoma virus (RSV) promoter, SV40 early/late promoters and the beta-actin promoter.

In preferred embodiments, the promoter may also comprise a regulatable sequence such as the Tet operator sequence. This would be extremely useful, for example, in cases where the gene products are effecting a result other than that desired and repression is sought.

Preferred transcription termination sequences present within the gene expression cassette are the bovine growth hormone terminator/polyadenylation signal (bGHpA) and the short synthetic polyA signal (SPA) of 50 nucleotides in length, defined as follows: AATAAAAGATCTTTATTTTCATTAGATCTGTGTGTTGGT-TTTTGTGTG (SEQ ID NO:26).

The combination of the CMV promoter (devoid of the intron A region) with the BGH terminator is particularly preferred although other promoter/terminator combinations in the context of FG adenovirus may also be used.

Other embodiments incorporate a leader or signal peptide into the transgene. A preferred leader is that from the tissue-specific plasminogen activator protein, tPA. Examples include but are not limited to the various tPA-gag, tPA-pol and tPA-nef adenovirus-based vaccines disclosed throughout this specification.

In view of the improved adenovirus vectors described herein, an essential portion of the present invention are adenoviral-based HIV vaccines comprising said adenovirus backbones which may be administered to a mammalian host, preferably a human host, in either a prophylactic or therapeutic setting. The HIV vaccines of the present invention, whether administered alone or in combination regimens with other viral- or non-viral-based DNA vaccines, should elicit potent and broad cellular immune responses against HIV that will either lessen the likelihood of persistent virus infection and/or lead to the establishment of a clinically significant lowered virus load



subject to HIV infection or in combination with HAART therapy, mitigate the effects of previously established HIV infection (antiviral immunotherapy(ARI)). While any HIV antigen (e.g., gag, pol, nef, gp160, gp41, gp120, tat, rev, etc.) may be utilized in the herein described recombinant adenoviral vectors, preferred embodiments include the codon optimized p55 gag antigen (herein exemplified as MRKAd5gag), pol and nef. Sequences based on different Clades of HIV-1 are suitable for use in the instant invention, most preferred of which are Clade B and Clade C. Particularly preferred embodiments are those sequences (especially, codon-optimized sequences) based on consensus Clade B sequences. Preferred versions of the MRKAd5pol and MRKAd5nef series of adenoviral vaccines will encode modified versions of pol or nef, as discussed herein. Preferred embodiments of the MRKAd5HIV-1 vectors carrying HIV envelope genes and modifications thereof comprise the HIV codon-optimized *env* sequences of PCT International Applications PCT/US97/02294 and PCT/US97/10517, published August 28, 1997 (WO 97/31115) and December 24, 1997, respectively; both documents of which are hereby incorporated by reference.

A most preferred aspect of the instant invention is the disclosed use of the adenoviral vector described above to effectuate expression of HIV gag. Sequences for many genes of many HIV strains are publicly available in GENBANK and primary, field isolates of HIV are available from the National Institute of Allergy and Infectious Diseases (NIAID) which has contracted with Quality Biological (Gaithersburg, MD) to make these strains available. Strains are also available from the World Health Organization (WHO), Geneva Switzerland. It is preferred that the gag gene be from an HIV-1 strain (CAM-1; Myers et al, eds. "Human Retroviruses and AIDS: 1995, IIA3-IIA19, which is hereby incorporated by reference). This gene closely resembles the consensus amino acid sequence for the clade B (North American/European) sequence. Therefore, it is within the purview of the skilled artisan to choose an appropriate nucleotide sequence which encodes a specific HIV gag antigen, or immunologically relevant portion thereof. As shown in Example 25, a clade B or clade C based p55 gag antigen will potentially be useful on a global scale. As noted herein, the transgene of choice for insertion in to a DNA or MRKAd-based adenoviral vector of the present invention is a codon optimized version of p55 gag. Such a MRKAd5gag adenoviral vector is documented in Example 11 and is at least referred to herein as MRKAd5HIV-1gag. Of course, additional versions are contemplated, including but not limited to modifications such as promoter (e.g., mCMV for hCMV) and/or pA-terminations signal (SPA for bGH) switching, as well as generating MRK Ad5 backbones with or without deletion of the Ad5 E3 gene.

The present invention also relates a series of MRKAd5pol-based adenoviral vaccines which are shown herein to generate cellular immune responses subsequent to administration in mice and non-human primate studies. Several of the MRKAd5pol series are exemplified herein. One such adenoviral vector is referred to as

5 MRKAd5hCMV-inact opt pol(E3+), which comprises the MRKAd5 backbone, the hCMV promoter (no intron A), an inactivated pol transgene, and contains the Ad5 E3 gene in the adenoviral backbone. A second exemplified pre-adenovirus plasmid and concomitant virus is referred to as MRKAd5hCMV-inact opt pol(E3-), which is identical to the former adenoviral vector except that the E3 is deleted. Both

10 constructions contain a codon optimized, inactivated version of HIV-1 Pol, wherein at least the entire coding region is disclosed herein as SEQ ID NO:3 and the expressed protein is shown as SEQ ID NO:4 (see also Figure 17A-C and Table 1, which show targeted deletion for inactivated pol. This and other preferred codon optimized versions of HIV Pol as disclosed herein are essentially as described in U.S.

15 Application Serial No. 09/745,221, filed December 21, 2000 and PCT International Application PCT/US00/34724, also filed December 21, 2000, both documents which are hereby incorporated by reference. As disclosed in the above-mentioned documents, the open reading frame for these codon-optimized HIV-1 Pol-based DNA vaccines are represented by codon optimized DNA molecules encoding codon

20 optimized HIV-1 Pol (e.g. SEQ ID NO:2), codon optimized HIV-1 Pol fused to an amino terminal localized leader sequence (e.g. SEQ ID NO:6), and especially preferable, and exemplified by the MRKAd5-Pol construct in e.g., Example 19, biologically inactivated pol ("inact opt Pol"; e.g., SEQ ID NO:4) which is devoid of significant PR, RT, RNase or IN activity associated with wild type Pol. In addition, a

25 construct related to SEQ ID NO:4 is contemplated which contains a leader peptide at the amino terminal region of the IA Pol protein. A specific construct is ligated within an appropriate DNA plasmid vector containing regulatory regions operatively linked to the respective HIV-1 Pol coding region, with or without a nucleotide sequence encoding a functional leader peptide. To this end, various HIV-1 Pol constructs

30 disclosed herein relate to open reading frames for cloning to the enhanced first generation Ad vectors of the present invention (such a series of MRKAd5pol adenoviral vaccine vectors), including but not limited to wild type Pol (comprising the DNA molecule encoding WT opt Pol, as set forth in SEQ ID NO:2), tPA-opt WTPol, (comprising the DNA molecule encoding tPA Pol, as set forth in SEQ ID NO:6), inact

35 opt Pol (comprising the DNA molecule encoding IA Pol, as set forth in SEQ ID NO:4), and tPA-inact opt Pol, (comprising the DNA molecule encoding tPA-inact opt

Pol, as set forth in SEQ ID NO:8). The pol-based versions of enhanced first generation adenovirus vaccines elicit CTL and Th cellular immune responses upon administration to the host, including primates and especially humans. As noted in the above, an effect of the cellular immune-directed vaccines of the present invention should be a lower transmission rate to previously uninfected individuals and/or reduction in the levels of the viral loads within an infected individual, so as to prolong the asymptomatic phase of HIV-1 infection.

The present invention further relates to a series of MRKAd5nef-based adenoviral vaccines which, similar to HIV gag and pol antigens, generate cellular immune responses subsequent to administration in mice and non-human primate studies. The MRKAd5nef series are exemplified herein by utilizing the improved MRK adenoviral backbone in combination with modified versions of HIV nef. These exemplified MRKAd5nef vectors are as follows: (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+), which comprises the improved MRKAd5 backbone, a human CMV promoter an intact Ad5 E3 gene and a modified nef gene: (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+), which is the same as (1) above but substituting a murine CMV promoter for a human CMV promoter; and (3) MRKAd5mCMV-tpanef(LLAA) (E3+), which is the same as (2) except that the nef transgene is tpanef(LLAA). Codon optimized versions of HIV-1 Nef and HIV-1 Nef modifications are essentially as described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and PCT International Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference. Particular embodiments of codon optimized Nef and Nef modifications relate to a DNA molecule encoding HIV-1 Nef from the HIV-1 jf1 isolate wherein the codons are optimized for expression in a mammalian system such as a human. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:9, while the expressed open reading frame is disclosed herein as SEQ ID NO:10. Another embodiment of Nef-based coding regions for use in the adenoviral vectors of the present invention comprise a codon optimized DNA molecule encoding a protein containing the human plasminogen activator (tpa) leader peptide fused with the NH<sub>2</sub>-terminus of the HIV-1 Nef polypeptide. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:11, while the expressed open reading frame is disclosed herein as SEQ ID NO:12. Another modified Nef optimized coding region relates to a DNA molecule encoding optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175, herein

described as opt nef (G2A, LLAA). The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:13, while the expressed open reading frame is disclosed herein as SEQ ID NO:14. MRKAd5nef vectors (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+) and (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+) contain this transgene. An additional embodiment relates to a DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA), comprises an open reading frame which encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfr1), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175, herein referred to as opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, while the expressed open reading frame is disclosed herein as SEQ ID NO:16. The MRKAd5nef vector "MRKAd5mCMV-tpanef(LLAA) (E3+)" contains this transgene.

Along with the improved MRKAd5gag adenovirus vaccine vector described herein, generation of a MRKAd5pol and MRKAd5nef adenovirus vector provide for enhanced HIV vaccine capabilities. Namely, the generation of this trio of adenoviral vaccine vectors, all shown to generate effective cellular immune responses subsequent to host administration, provide for the ability to administer these vaccine candidates not only alone, but preferably as part of a divalent (i.e., gag and nef, gag and pol, or pol and nef components) or a trivalent vaccine (i.e., gag, pol and nef components). Therefore, a preferred aspect of the present invention are vaccine formulations and associated methods of administration and concomitant generation of host cellular immune responses associated with formulating three separate series of MRKAd5-based adenoviral vector vaccines. Of course, this MRKAd5 vaccine series based on distinct HIV antigens promotes expanded opportunities for formulation of a divalent or trivalent vaccine, or possibly administration of separate formulations of one or more monovalent or divalent formulations within a reasonable window of time. It is also within the scope of the present invention to embark on combined modality regimes which include multiple but distinct components from a specific antigen. An example, but certainly not a limitation, would be separate MRKAd5pol vectors, with one vaccine vector expressing wild type Pol (SEQ ID NO:2) and another MRKAd5pol vector expressing inactivated Pol (SEQ ID NO:6). Another example might be separate MRKAd5nef vectors, with one vaccine vector expressing the tPA/LLAA version of Nef (SEQ ID NO:16) and another MRKAd5nef vector expressing the G2A,LLAA modified version of Nef (SEQ ID NO:14). Therefore, the MRKAd5 adenoviral vectors of the present invention may be used in combination

with multiple, distinct HIV antigen classes. Each HIV antigen class is subject to sequence manipulation, thus providing for a multitude of potential vaccine combinations; and such combinations are within the scope of the present invention. The utilization of such combined modalities vaccine formulation and administration  
5 increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

The present invention also relates to application of a mono-, dual-, or tri-modality administration regime of the MRKAd5gag, pol and nef adenoviral vaccine series in a prime/boost vaccination schedule. This prime/boost schedule may include  
10 any reasonable combination of the MRKAd5gag, pol and nef adenoviral vaccine series disclosed herein. In addition, a prime/boost regime may also involve other viral and/or non-viral DNA vaccines. A preferable addition to an adenoviral vaccine vector regime includes but is not limited to plasmid DNA vaccines, especially DNA plasmid vaccines that contain at least one of the codon optimized gag, pol and nef  
15 constructions, as disclosed herein.

Therefore, one aspect of this invention is the administration of the adenoviral vector containing the optimized gag gene in a prime/boost regiment in conjunction with a plasmid DNA encoding gag. To distinguish this plasmid from the adenoviral-containing shuttle plasmids used in the construction of an adenovirus vector, this  
20 plasmid will be referred to as a "vaccine plasmid" or "DNA plasmid vaccine". Preferred vaccine plasmids for use in this administration protocol are disclosed in pending U.S. patent application 09/017,981, filed February 3, 1998 and WO98/34640, published August 13, 1998, both of which are hereby incorporated by reference. Briefly, the preferred vaccine plasmid is designated V1Jns-FLgag, which expresses  
25 the same codon-optimized gag gene as the adenoviral vectors of this invention (see Figure 2 for the nucleotide sequence of the exemplified optimized codon version of full length p55 gag). The vaccine plasmid backbone, designated V1Jns contains the CMV immediate-early (IE) promoter and intron A, a bovine growth hormone-derived polyadenylation and transcription termination sequence as the gene expression  
30 regulatory elements, and a minimal pUC backbone; see Montgomery *et al.*, 1993, *DNA Cell Biol.* 12:777-783. The pUC sequence permits high levels of plasmid production in *E. coli* and has a neomycin resistance gene in place of an ampicillin resistance gene to provide selected growth in the presence of kanamycin. Alternatively, a vaccine plasmid which has the CMV promoter deleted of intron A can  
35 be used. Those of skill in the art will recognize that alternative vaccine plasmid

vectors may be easily substituted for these specific constructs, and this invention specifically envisions use of such alternative plasmid DNA vaccine vectors.

Another aspect of the present invention is a prime/boost regimen which includes a vaccine plasmid which encodes an HIV pol antigen, preferably a codon optimized form of pol and also preferably a vaccine plasmid which comprises a  
5 nucleotide sequence which encodes a Pol antigen selected from the group of Pol antigens as shown in SEQ ID NOs: 2, 4, 6 and 8. The variety of potential DNA plasmid vaccines which encode various biologically active forms of HIV-1 Pol, wherein administration, intracellular delivery and expression of the HIV-1 Pol gene of  
10 interest elicits a host CTL and Th response. The preferred synthetic DNA molecules of the present invention encode codon optimized wild type Pol (without Pro activity) and various codon optimized inactivated HIV-1 Pol proteins. The HIV-1 *pol* open reading disclosed herein are especially preferred for pharmaceutical uses, especially for human administration as delivered via a recombinant adenoviral vaccine,  
15 especially an enhanced first generation recombinant adenoviral vaccine as described herein. Several embodiments of this portion of the invention are provided in detail below, namely DNA molecules which comprise a HIV-1 pol open reading frame, whether encoding full length pol or a modification or fusion as described herein, wherein the codon usage has been optimized for expression in a mammal, especially a  
20 human. Again, these DNA sequences are positioned appropriately within a recombinant adenoviral vector, such as the exemplified recombinant adenoviral vector described herein, so as to promote expression of the respective HIV-1 Pol gene of interest, and subsequent to administration, elicit a host CTL and Th response. Again, these preferred, but in no way limiting, pol genes are as disclosed herein and  
25 essentially as described in U.S. Application Serial No. 09/745,221, filed December 21, 2000 and PCT International Application PCT/US00/34724, also filed December 21, 2000, both documents which are hereby incorporated by reference.

A third series of vaccine plasmids which are useful in a combined modality and/or prime/boost regimen are vaccine plasmids which encode an HIV nef antigen or  
30 biologically and/or immunologically relevant modification thereof. As noted elsewhere, preferred vaccine plasmids contain a codon optimized form of nef and also preferably comprise a nucleotide sequence which encodes a Nef antigen selected from the group of Nef antigens as shown in SEQ ID NOs: 10, 12, 14 and 16. These preferred nef coding regions are disclosed herein, as well as being described in U.S.  
35 Application Serial No. 09/738,782, filed December 15, 2000 and PCT International

Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference.

Therefore, the adenoviral vaccines and plasmid DNA vaccines of this invention may be administered alone, or may be part of a prime and boost administration regimen. A mixed modality priming and booster inoculation scheme will result in an enhanced immune response, particularly if pre-existing anti-vector immune responses are present. This one aspect of this invention is a method of priming a subject with the plasmid vaccine by administering the plasmid vaccine at least one time, allowing a predetermined length of time to pass, and then boosting by administering the adenoviral vaccine. Multiple primings typically, 1-4, are usually employed, although more may be used. The length of time between priming and boost may typically vary from about four months to a year, but other time frames may be used. In experiments with rhesus monkeys, the animals were primed four times with plasmid vaccines, then were boosted 4 months later with the adenoviral vaccine. Their cellular immune response was notably higher than that of animals which had only received adenoviral vaccine. The use of a priming regimen may be particularly preferred in situations where a person has a pre-existing anti-adenovirus immune response.

Furthermore and in the alternative, multiple HIV-1 viral antigens, such as the MRKAd5 adenoviral vaccines disclosed herein, may be ligated into a proper shuttle plasmid for generation of a pre-adenoviral plasmid comprising multiple open reading frames. For example a trivalent vector may comprise a gag-pol-nef fusion, in either a E3(-) or E3(+) background, preferably a E3 deleted backbone, or possibly a "2+1" divalent vaccine, such as a gag-pol fusion (i.e., codon optimized p55 gag and inactivated optimized pol; Example 29 and Table 25) within the same MRKAd5 backbone, with each open reading frame being operatively linked to a distinct promoter and transcription termination sequence. Alternatively, the two open reading frames may be operatively linked to a single promoter, with the open reading frames operatively linked by an internal ribosome entry sequence (IRES), as disclosed in International Publication No. WO 95/24485, which is hereby incorporated by reference. Figure 9 shows that the use of multiple promoters and termination sequences provide for similar growth properties, while Figure 28 shows that these MRKAd5gag-based vectors are also stable at least through passage 21. In the absence of the use of IRES-based technology, it is preferred that a distinct promoter be used to support each respective open reading frame, so as to best preserve vector stability. As examples, and certainly not as limitations, potential multiple transgene vaccines may

include a three transgene vector such as hCMV-gagpol-bGHpA + mCMV-nef-SPA in an E3 deleted backbone or hCMV-gagpol-bGHpA + mCMV-nef-SPA(E3+). Potential "2+1" divalent vaccines of the present invention might be a hCMV-gag-bGHpA + mCMV-nef-SPA in an E3+ backbone (vector #1) in combination with

5 hCMV-pol-bGHpA in an E3+ backbone (vector #2), with all transgenes in the E1 parallel orientation. Fusion constructs other than the gag-pol fusion described above are also suitable for use in various divalent vaccine strategies and can be composed of any two HIV antigens fused to one another (*e.g.*, nef-pol and gag-nef). These adenoviral compositions are, as above, preferably delivered along with an adenoviral

10 composition comprising an additional HIV antigen in order to diversify the immune response generated upon administration. Therefore, a multivalent vaccine delivered in a single, or possible second, adenoviral vector is certainly contemplated as part of the present invention. Again, this mode of administration is another example of whereby an efficacious adenovirus-based HIV-1 vaccine may be administered via a

15 combined modality regime. It is important to note, however, that in terms of deciding on an insert for the disclosed adenoviral vectors, due consideration must be dedicated to the effective packaging limitations of the adenovirus vehicle. Adenovirus has been shown to exhibit an upper cloning capacity limit of approximately 105% of the wildtype Ad5 sequence.

20 Regardless of the gene chosen for expression, it is preferred that the sequence be "optimized" for expression in a human cellular environment. A "triplet" codon of four possible nucleotide bases can exist in 64 variant forms. That these forms provide the message for only 20 different amino acids (as well as transcription initiation and termination) means that some amino acids can be coded for by more than one codon.

25 Indeed, some amino acids have as many as six "redundant", alternative codons while some others have a single, required codon. For reasons not completely understood, alternative codons are not at all uniformly present in the endogenous DNA of differing types of cells and there appears to exist variable natural hierarchy or "preference" for certain codons in certain types of cells. As one example, the amino

30 acid leucine is specified by any of six DNA codons including CTA, CTC, CTG, CTT, TTA, and TTG (which correspond, respectively, to the mRNA codons, CUA, CUC, CUG, CUU, UUA and UUG). Exhaustive analysis of genome codon frequencies for microorganisms has revealed endogenous DNA of *E. coli* most commonly contains the CTG leucine-specifying codon, while the DNA of yeasts and slime molds most

35 commonly includes a TTA leucine-specifying codon. In view of this hierarchy, it is generally held that the likelihood of obtaining high levels of expression of a leucine-



rich polypeptide by an *E. coli* host will depend to some extent on the frequency of codon use. For example, a gene rich in TTA codons will in all probability be poorly expressed in *E. coli*, whereas a CTG rich gene will probably highly express the polypeptide. Similarly, when yeast cells are the projected transformation host cells for expression of a leucine-rich polypeptide, a preferred codon for use in an inserted DNA would be TTA.

The implications of codon preference phenomena on recombinant DNA techniques are manifest, and the phenomenon may serve to explain many prior failures to achieve high expression levels of exogenous genes in successfully transformed host organisms--a less "preferred" codon may be repeatedly present in the inserted gene and the host cell machinery for expression may not operate as efficiently. This phenomenon suggests that synthetic genes which have been designed to include a projected host cell's preferred codons provide a preferred form of foreign genetic material for practice of recombinant DNA techniques. Thus, one aspect of this invention is an adenovirus vector or adenovirus vector in some combination with a vaccine plasmid where both specifically include a gene which is codon optimized for expression in a human cellular environment. As noted herein, a preferred gene for use in the instant invention is a codon-optimized HIV gene and, particularly, HIV gag, pol or nef.

Adenoviral vectors in accordance with the instant invention can be constructed using known techniques, such as those reviewed in Hitt et al, 1997 "Human Adenovirus Vectors for Gene Transfer into Mammalian Cells" *Advances in Pharmacology* 40:137-206, which is hereby incorporated by reference.

In constructing the adenoviral vectors of this invention, it is often convenient to insert them into a plasmid or shuttle vector. These techniques are known and described in Hitt et al., *supra*. This invention specifically includes both the adenovirus and the adenovirus when inserted into a shuttle plasmid.

Preferred shuttle vectors contain an adenoviral portion and a plasmid portion. The adenoviral portion is essentially the same as the adenovirus vector discussed *supra*, containing adenoviral sequences (with non-functional or deleted E1 and E3 regions) and the gene expression cassette, flanked by convenient restriction sites. The plasmid portion of the shuttle vector often contains an antibiotic resistance marker under transcriptional control of a prokaryotic promoter so that expression of the antibiotic does not occur in eukaryotic cells. Ampicillin resistance genes, neomycin resistance genes and other pharmaceutically acceptable antibiotic resistance markers may be used. To aid in the high level production of the polynucleotide by

fermentation in prokaryotic organisms, it is advantageous for the shuttle vector to contain a prokaryotic origin of replication and be of high copy number. A number of commercially available prokaryotic cloning vectors provide these benefits. It is desirable to remove non-essential DNA sequences. It is also desirable that the vectors not be able to replicate in eukaryotic cells. This minimizes the risk of integration of polynucleotide vaccine sequences into the recipients' genome. Tissue-specific promoters or enhancers may be used whenever it is desirable to limit expression of the polynucleotide to a particular tissue type.

In one embodiment of this invention, the pre-plasmids (e.g., pMRKAd5pol, pMRKAd5nef and pMRKAd5gag) were generated by homologous recombination using the MRKHVE3 (and MRKHVO for the E3- version) backbones and the appropriate shuttle vector, as shown for pMRKAd5pol in Figure 22 and for pMRKAd5nef in Figure 23. The plasmid in linear form is capable of replication after entering the PER.C6<sup>®</sup> cells and virus is produced. The infected cells and media were harvested after viral replication was complete.

Viral vectors can be propagated in various E1 complementing cell lines, including the known cell lines 293 and PER.C6<sup>®</sup>. Both these cell lines express the adenoviral E1 gene product. PER.C6<sup>®</sup> is described in WO 97/00326 (published January 3, 1997) and issued U.S. Patent No. 6,033,908, both of which are hereby incorporated by reference. It is a primary human retinoblast cell line transduced with an E1 gene segment that complements the production of replication deficient (FG) adenovirus, but is designed to prevent generation of replication competent adenovirus by homologous recombination. Cells of particular interest have been stably transformed with a transgene that encodes the AD5E1A and E1B gene, like PER.C6<sup>®</sup>, from 459 bp to 3510 bp inclusive. 293 cells are described in Graham et al., 1977 *J. Gen. Virol* 36:59-72, which is hereby incorporated by reference. As stated above, consideration must be given to the adenoviral sequences present in the complementing cell line used. It is important that the sequences not overlap with that present in the vector if the possibility of recombination is to be minimized.

It has been found that vectors generated in accordance with the above description are more effective in inducing an immune response and, thus, constitute very promising vaccine candidates. More particularly, it has been found that first generation adenoviral vectors in accordance with the above description carrying a codon-optimized HIV gag gene, regulated with a strong heterologous promoter can be used as human anti-HIV vaccines, and are capable of inducing immune responses.

Standard techniques of molecular biology for preparing and purifying DNA constructs enable the preparation of the DNA immunogens of this invention.

A vaccine composition comprising an adenoviral vector in accordance with the instant invention may contain physiologically acceptable components, such as  
5 buffer, normal saline or phosphate buffered saline, sucrose, other salts and polysorbate. One preferred formulation has: 2.5-10 mM TRIS buffer, preferably about 5 mM TRIS buffer; 25-100 mM NaCl, preferably about 75 mM NaCl; 2.5-10% sucrose, preferably about 5% sucrose; 0.01 -2 mM  $MgCl_2$ ; and 0.001%-0.01% polysorbate 80 (plant derived). The pH should range from about 7.0-9.0, preferably  
10 about 8.0. One skilled in the art will appreciate that other conventional vaccine excipients may also be used it make the formulation. The preferred formulation contains 5mM TRIS, 75 mM NaCl, 5% sucrose, 1mM  $MgCl_2$ , 0.005% polysorbate 80 at pH 8.0 This has a pH and divalent cation composition which is near the optimum for Ad5 stability and minimizes the potential for adsorption of virus to a glass surface.  
15 It does not cause tissue irritation upon intramuscular injection. It is preferably frozen until use.

The amount of adenoviral particles in the vaccine composition to be introduced into a vaccine recipient will depend on the strength of the transcriptional and translational promoters used and on the immunogenicity of the expressed gene  
20 product. In general, an immunologically or prophylactically effective dose of  $1 \times 10^7$  to  $1 \times 10^{12}$  particles and preferably about  $1 \times 10^{10}$  to  $1 \times 10^{11}$  particles is administered directly into muscle tissue. Subcutaneous injection, intradermal introduction, impression through the skin, and other modes of administration such as intraperitoneal, intravenous, or inhalation delivery are also contemplated. It is also  
25 contemplated that booster vaccinations are to be provided. Following vaccination with HIV adenoviral vector, boosting with a subsequent HIV adenoviral vector and/or plasmid may be desirable. Parenteral administration, such as intravenous, intramuscular, subcutaneous or other means of administration of interleukin-12 protein, concurrently with or subsequent to parenteral introduction of the vaccine  
30 compositions of this invention is also advantageous.

The adenoviral vector and/or vaccine plasmids of this invention polynucleotide may be unassociated with any proteins, adjuvants or other agents which impact on the recipients' immune system. In this case, it is desirable for the vector to be in a physiologically acceptable solution, such as, but not limited to, sterile  
35 saline or sterile buffered saline. Alternatively, the vector may be associated with an adjuvant known in the art to boost immune responses (i.e., a "biologically effective"

adjuvant), such as a protein or other carrier. Vaccine plasmids of this invention may, for instance, be delivered in saline (e.g., PBS) with or without an adjuvant. Preferred adjuvants are Alum or CRL1005 Block Copolymer. Agents which assist in the cellular uptake of DNA, such as, but not limited to, calcium ions, may also be used to  
5 advantage. These agents are generally referred to herein as transfection facilitating reagents and pharmaceutically acceptable carriers. Techniques for coating microprojectiles coated with polynucleotide are known in the art and are also useful in connection with this invention.

This invention also includes a prime and boost regimen wherein a first  
10 adenoviral vector is administered, then a booster dose is given. The booster dose may be repeated at selected time intervals. Alternatively, a preferred inoculation scheme comprises priming with a first adenovirus serotype and then boosting with a second adenovirus serotype. More preferably, the inoculation scheme comprises priming with a first adenovirus serotype and then boosting with a second adenovirus serotype,  
15 wherein the first and second adenovirus serotypes are classified within separate subgroups of adenoviruses. The above prime/boost schemes are particularly preferred in those situations where a preexisting immunity is identified to the adenoviral vector of choice. In this type of scheme, the individual or population of individuals is primed with an adenovirus of a serotype other than that to which the preexisting  
20 immunity is identified. This enables the first adenovirus to effectuate sufficient expression of the transgene while evading existing immunity to the second adenovirus (the boosting adenovirus) and, further, allows for the subsequent delivery of the transgene via the boosting adenovirus to be more effective. Adenovirus serotype 5 is one example of a virus to which such a scheme might be desirable. In accordance  
25 with this invention, therefore, one might decide to prime with a non-group C adenovirus (e.g., Ad12, a group A adenovirus, Ad24, a group D adenovirus, or Ad35, a group B adenovirus) to evade anti-Ad5 immunity and then boost with Ad5, a group C adenovirus. Another preferred embodiment involves administration of a different adenovirus (including non-human adenovirus) vaccine followed by administration of  
30 the adenoviral vaccines disclosed. In the alternative, a viral antigen of interest can be first delivered via a viral vaccine other than an adenovirus-based vaccine, and then followed with the adenoviral vaccine disclosed. Alternative viral vaccines include but are not limited to pox virus and venezuelan equine encephalitis virus.

A large body of human and animal data supports the importance of cellular  
35 immune responses, especially CTL in controlling (or eliminating) HIV infection. In humans, very high levels of CTL develop following primary infection and correlate

with the control of viremia. Several small groups of individuals have been described who are repeatedly exposed to HIV by remain uninfected; CTL has been noted in several of these cohorts. In the SIV model of HIV infection, CTL similarly develops following primary infection, and it has been demonstrated that addition of anti-CD8 monoclonal antibody abrogated this control of infection and leads to disease progression. This invention uses adenoviral vaccines alone or in combination with plasmid vaccines to induce CTL.

The following non-limiting Examples are presented to better illustrate the invention.

#### EXAMPLE 1

##### Removal of the Intron A Portion of the hCMV Promoter

GMP grade pVJnsHIVgag was used as the starting material to amplify the hCMV promoter. PVIJnsHIVgag is a plasmid comprising the CMV immediate-early (IE) promoter and intron A, a full-length codon-optimized HIV gag gene, a bovine growth hormone-derived polyadenylation and transcriptional termination sequence, and a minimal pUC backbone; see Montgomery *et al.*, *supra* for a description of the plasmid backbone. The amplification was performed with primers suitably positioned to flank the hCMV promoter. A 5' primer was placed upstream of the *MscI* site of the hCMV promoter and a 3' primer (designed to contain the *BglII* recognition sequence) was placed 3' of the hCMV promoter. The resulting PCR product (using high fidelity *Taq* polymerase) which encompassed the entire hCMV promoter (minus intron A) was cloned into TOPO PCR blunt vector and then removed by double digestion with *MscI* and *BglII*. This fragment was then cloned back into the original GMP grade pV1JnsHIVgag plasmid from which the original promoter, intron A, and the gag gene were removed following *MscI* and *BglII* digestion. This ligation reaction resulted in the construction of a hCMV promoter (minus intron A) + bGHpA expression cassette within the original pV1JnsHIVgag vector backbone. This vector is designated pVJnsCMV(no intron).

The FLgag gene was excised from pV1JnsHIVgag using *BglII* digestion and the 1,526 bp gene was gel purified and cloned into pV1JnsCMV(no intron) at the *BglII* site. Colonies were screened using *SmaI* restriction enzymes to identify clones that carried the FLgag gene in the correct orientation. This plasmid, designated pV1JnsCMV(no intron)-FLgag-bGHpA, was fully sequenced to confirm sequence integrity.

Two additional transgenes were also constructed. The plasmid, pV1JnsCMV(no intron)-FLgag-SPA, is identical to pV1JnsCMV(no intron)-FLgag-bGHpA except that the bovine growth hormone polyadenylation signal has been replaced with a short synthetic polyA signal (SPA) of 50 nucleotides in length. The sequence of the SPA is as shown, with the essential components (poly(A) site, (GT)<sub>n</sub>, and (T)<sub>n</sub>; respectively) underlined:

AATAAAAGATCTTTATTTTCATTAGATCTGTGTG TTGGTTTTTTGTGTG  
(SEQ ID NO:18).

The plasmid, pV1Jns-mCMV-FLgag-bGHpA, is identical to the pV1JnsCMV(no intron)-FLgag-bGHpA except that the hCMV promoter has been removed and replaced with the murine CMV (mCMV) promoter.

Figure 3 diagrammatically shows the new transgene constructs in comparison with the original transgene.

## EXAMPLE 2

### Gag Expression Assay for Modified Gag Transgenes

Gag Elisa was performed on culture supernatants obtained from transient tissue culture transfection experiments in which the two new hCMV-containing plasmid constructs, pV1JnsCMV(no intron)-FLgag-bGHpA and pV1JnsCMV(no intron)-FLgag-SPA, both devoid of intron A, were compared to pV1JnsHIVgag which, as noted above possesses the intron A as part of the hCMV promoter. Table 2 below shows the *in vitro* gag expression data of the new gag plasmids compared with the GMP grade original plasmid. The results displayed in Table 2 show that both of the new hCMV gag plasmid constructs have expression capacities comparable to the original plasmid construct which contains the intron A portion of the hCMV promoter.

Table 2: *In vitro* DNA transfection of original and new plasmid HIV-1 gag constructs.

Plasmid	$\mu\text{g gag}/10^6 \text{ COS cells}/5\mu\text{g DNA}/48 \text{ hr}$
HIVFL-gagPR9901 <sup>a</sup>	10.8
PV1Jns-hCMV-FLgag-bGHpA <sup>b</sup>	16.6
pV1Jns-hCMV-FLgag-SPA <sup>b,c</sup>	12.0

<sup>a</sup> GMP grade pV1Jns-hCMVintronA-FLgag-bGHpA.

5    <sup>b</sup> New plasmid constructions that have the intron A portion removed from the hCMV promoter.

<sup>c</sup> In this construct the bGH terminator has been replaced with the short synthetic polyadenylation signal (SPA)

10

## EXAMPLE 3

## Rodent (Balb/c) Study for Modified gag Transgenes

A rodent study was performed on the two new plasmid constructs described above – pV1JnsCMV(no intron)-FLgag-bGHpA and pV1JnsCMV(no intron)-FLgag-SPA - in order to compare them with the construct described above  
 15    possessing the intron A portion of the CMV promoter, pV1JnsHIVgag. Gag antibody and Elispot responses (described in PCT International Application No. PCT/US00/18332 (WO 01/02607) filed July 3, 2000, claiming priority to U.S. Provisional Application Serial No. 60/142,631, filed July 6, 1999 and U.S. Application Serial No. 60/148,981, filed August 13, 1999, all three applications which  
 20    are hereby incorporated by reference) were measured. The results displayed in Table 3 below, show that the new plasmid constructs behaved equivalently to the original construct in Balb/c mice with respect to their antibody and T-cell responses at both dosages of plasmid DNA tested, 20  $\mu\text{g}$  and 200  $\mu\text{g}$ .

## EXAMPLE 4

**Table 3:** HIV191: Immunogenicity of V1Jns-gag under different promoter and termination control elements.

DNA <sup>a</sup> Promoter/terminator	Dose, ug <sup>b</sup>	Anti-p24 Titers (3 Wk PD1) <sup>c</sup>			SFC/10 <sup>6</sup> Cells (4 Wk PD1) <sup>d</sup>		
		GMT	+SE	-SE	Media	gag197-205	p24
HIVFL-gagPR9901 (GMP grade)	200	12800	4652	3412	2(2)	129(19)	30(11)
	20	5572	1574	1227	0	56(9)	25(6)
pV1Jns-hCMV- FL-gag-bGHpA	200	11143	2831	2257	0	98(5)	12(6)
	20	7352	2808	2032	0	73(9)	11(6)
pV1Jns-hCMV- FL-gag-SPA	200	16890	5815	4326	1(1)	94(4)	26(7)
	20	5971	5361	2825	0	85(17)	38(10)
Naïve	0	123	50	38	0	0	0

<sup>a</sup>in PBS<sup>b</sup>i.m. Injections into both quads, 50 µL per quad<sup>c</sup>n=10; GMT, geometric mean titer; SE, standard. error<sup>d</sup>n=5, pooled spleens; mean of triplicate wells and standard. deviation. in parentheses;

## Construction of the Modified Shuttle Vector - "MRKpdeIE1 Shuttle"

The modifications to the original Ad5 shuttle vector (pdeIE1sp1A; a vector comprising Ad5 sequences from basepairs 1-341 and 3524-5798, with a multiple cloning region between nucleotides 341 and 3524 of Ad5, included the following three manipulations carried out in sequential cloning steps as follows:

(1) The left ITR region was extended to include the *PacI* site at the junction between the vector backbone and the adenovirus left ITR sequences. This allow for easier manipulations using the bacterial homologous recombination system.

(2) The packaging region was extended to include sequences of the wild-type (WT) adenovirus from 342 bp to 450 bp inclusive.

(3) The area downstream of pIX was extended 13 nucleotides (i.e., nucleotides 3511-3523 inclusive).

These modifications (Figure 4) effectively reduced the size of the E1 deletion without overlapping with any part of the E1A/E1B gene present in the transformed PER.C6<sup>®</sup> cell line. All manipulations were performed by modifying the Ad shuttle vector pdeIE1sp1A.

Once the modifications were made to the shuttle vector, the changes were incorporated into the original Ad5 adenovector backbones (pAdHVO and pAdHVE3) by bacterial homologous recombination using *E. coli* BJ5183 chemically competent cells.



## EXAMPLE 5

Construction of Modified Adenovector Backbones (E3+ and E3-)

The original adenovectors pAdHVO (comprising all Ad5 sequences except those nucleotides encompassing the E1 and E3 regions ) and pAdHVE3 (comprising all Ad5 sequences except those nucleotides encompassing the E1 region), were each reconstructed so that they contained the modifications to the E1 region. This was accomplished by digesting the newly modified shuttle vector (MRKpdelE1 shuttle) with *Pac*I and *Bst*Z1101 and isolating the 2,734 bp fragment which corresponds to the adenovirus sequence. This fragment was co-transformed with DNA from either *Cla*I linearized pAdHVO (E3- adenovector) or *Cla*I linearized pAdHVE3 (E3+adenovector) into *E. coli* BJ5183 competent cells. At least two colonies from each transformation were selected and grown in Terrific™ broth for 6-8 hours until turbidity was reached. DNA was extracted from each cell pellet and then transformed into *E. coli* XL1 competent cells. One colony from each transformation was selected and grown for plasmid DNA purification. The plasmid was analyzed by restriction digestions to identify correct clones. The modified adenovectors were designated MRKpAdHVO (E3- plasmid) and MRKpAdHVE3 (E3+ plasmid). Virus from these new adenovectors (MRKHVO and MRKHVE3, respectively) as well as the old version of the adenovectors were generated in the PER.C6® cell lines to accommodate the following series of viral competition experiments. In addition, the multiple cloning site of the original shuttle vector contained *Cla*I, *Bam*HI, *Xho*I, *Eco*RV, *Hind*III, *Sal*I, and *Bgl*II sites. This MCS was replaced with a new MCS containing *Not*I, *Cla*I, *Eco*RV and *Asc*I sites. This new MCS has been transferred to the MRKpAdHVO and MRKpAdHVE3 pre-plasmids along with the modification made to the packaging region and pIX gene.

## EXAMPLE 6

Analysis of the Effect of the Packaging Signal Extension

To study the effects of the modifications made to the E1 deletion region, the viruses obtained from the original backbone (pAdHVE3) and the new backbone (MRKpAdHVE3) were mixed together in equal MOI ratios (1:1 and 5:5) and passaged through several rounds; see Figure 5, Expt.#1. Both of the viruses in the experiment contained the E3 gene intact and did not contain a transgene. The only difference between the two viruses was within the region of the E1 deletion. Following the coinfection of the viruses at P1 (passage 1), the mixtures were propagated through an additional 4 passages at which time the cells were harvested

and the virus extracted and purified by CsCl banding. The viral DNA was extracted and digested with *HindIII* and the digestion products were then radioactively labeled. For the controls, the respective pre-plasmids (pAdHVE3 ("OLD E3+"); MRKpAdHVE3 ("NEW E3+")) were also digested with *HindIII* (and *Pac1* to remove the vector backbone) and subsequently labeled with [<sup>33</sup>P]dATP. The radioactively labeled digestion products were subjected to gel electrophoresis and the gel was dried down onto Whatman paper before being exposed to autoradiographic film. Figure 6 clearly shows that the new adenovirus which has the addition made to the packaging signal region has a growth advantage compared with the original adenovirus. In the experiments performed (at either ratio tested), only the digestion bands pertaining to the newly modified virus were present. The diagnostic band of size 3,206 (from the new virus) was clearly present. However, there was no evidence of the diagnostic band of size 2,737 bp expected from the original virus.

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## EXAMPLE 7

Analysis of the Effect of the E3 Gene

The second set of the virus competition study involved mixing equal MOI ratio (1:1) of the newly modified viruses, that obtained from MRKpAdHVO and MRKpAdHVE3 (Figure 5, Expt. #2). In this set, both viruses had the new modifications made to the E1 deletion. The first virus (that from MRKpAdHVO) does not contain an E3 gene. The second virus (that from MRKpAdHVE3) does contain the E3 gene. Neither of the viruses contain a transgene. Following co-infection of the viruses, the mixtures were propagated through an additional 4 passages at which time the cells were harvested and the total virus extracted and purified by CsCl banding. The viral DNA was extracted and digested with *HindIII* and the digestion products were then radioactively labeled. For the controls, the respective pre-plasmids MRKpAdHVO ("NEW E3-"); MRKpAdHVE3 ("NEW E3+") were also digested with *HindIII* (and *Pac1* to remove the vector backbone) and then labeled with [<sup>33</sup>P]dATP. The radioactively labeled digestion products were subjected to gel electrophoresis and the gel was dried down onto Whatman paper before being exposed to autoradiographic film. Figure 6 shows the results of the viral DNA analysis of the E3+ virus and E3- virus mixing experiment. The diagnostic band corresponding to the E3+ virus (5,665 bp) was present in greater amount compared with the diagnostic band of 3,010 bp corresponding to the E3- virus. This indicates that the virus that contains the E3 gene is able to amplify more rapidly

compared with the virus that does not contain an E3 gene. This increased amplification capacity has been confirmed by growth studies; see Table 4 below.

### EXAMPLE 8

5 Construction of the new shuttle vector containing modified gag transgene –  
“MRKpdeIE1-CMV(no intron)-FLgag-bGHpA”

The modified plasmid pV1JnsCMV(no intron)-FLgag-bGHpA was digested with *MscI* overnight and then digested with *SfiI* for 2 hours at 50°C. The DNA was then treated with Mungbean nuclease for 30 mins at 30°C. The DNA mixture was desalted using the Qiaex II kit and then Klenow treated for 30 mins at 37°C to fully blunt the ends of the transgene fragment. The 2,559 bp transgene fragment was then gel purified. The modified shuttle vector (MRKpdeIE1 shuttle) was linearized by digestion with *EcoRV*, treated with calf intestinal phosphatase and the resulting 6,479 bp fragment was then gel purified. The two purified fragments were then ligated together and several dozen clones were screened to check for insertion of the transgene within the shuttle vector. Diagnostic restriction digestion was performed to identify those clones carrying the transgene in the E1 parallel and E1 anti-parallel orientation. This strategy was followed to clone in the other gag transgenes in the MRKpdeIE1 shuttle vector.

### EXAMPLE 9

### Construction of the MRK FG Adenovectors

The shuttle vector containing the HIV-1 gag transgene in the E1 parallel orientation, MRKpdeIE1-CMV(no intron)-FLgag-bGHpA, was digested with *Pac*1. The reaction mixture was digested with *Bsf*Z171. The 5,291 bp fragment was purified by gel extraction. The MRKpAdHVE3 plasmid was digested with *Cla*1 overnight at 37°C and gel purified. About 100 ng of the 5,290 bp shuttle +transgene fragment and ~100 ng of linearized MRKpAdHVE3 DNA were co-transformed into *E. coli* BJ5183 chemically competent cells. Several clones were selected and grown in 2 ml Terrific™ broth for 6-8 hours, until turbidity was reached. The total DNA from the cell pellet was purified using Qiagen alkaline lysis and phenol chloroform method. The DNA was precipitated with isopropanol and resuspended in 20 µl dH<sub>2</sub>O. A 2 µl aliquot of this DNA was transformed into *E. coli* XL-1 competent cells. A single colony from each separate transformation was selected and grown overnight in 3 ml LB +100 µg/ml ampicillin. The DNA was isolated using Qiagen columns. A positive clone was identified by digestion with the restriction enzyme *Bst*EII which cleaves

within the gag gene as well as the plasmid backbone. The pre-plasmid clone is designated MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA and is 37,498 bp in size. This strategy was followed to generate E3- and E3+ versions of each of the other gag transgene constructions in both E1 parallel and E1 anti-parallel versions. Figures 7A, 7B and 7C show the various combinations of adenovectors constructed.

## EXAMPLE 10

### Plasmid Competition Studies

A series of plasmid competition studies was carried out. Briefly, the screening of the various combinations of new constructs was performed by mixing equal amounts of each of two competing plasmids. In the experiment shown in Figure 8A, plasmids containing the same transgene but in different orientations were mixed together to create a "competition" between the two plasmids. The aim was to look at the effects of transgene orientation. In the experiment shown in Figure 8B, plasmids containing different polyadenylation signals (but in the same orientation) were mixed together in equal amounts. The aim was to assess effects of polyA signals. Following the initial transfection, the virus was passaged through ten rounds and the viral DNA analyzed by radioactive restriction analysis.

Analysis of the viral species from the plasmid mixing experiment (Figure 8A) showed that adenovectors which had the transgene inserted in the E1 parallel orientation amplified better and were able to out-compete the adenovirus which had the transgene inserted in the E1 anti-parallel orientation. Viral DNA analysis of the mixtures at passage 3 and certainly at passage 6, showed a greater ratio of the virus carrying the transgene in the E1 parallel orientation compared with the E1 antiparallel version. By passage 10, the only viral species observed was the adenovector with the transgene in the E1 parallel orientation for both transgenes tested (hCMV(no intron)-FLgag-bGHpA and hCMV(no intron)-FLgag-SPA).

Analysis of the viral species from the plasmid mixing experiment #2 (Figure 8B) at passages 3 and 6 showed that the polyadenylation signals tested (bGHpA and SPA) did not have an effect on the growth of the virus. Even at passage 10 the two viral species in the mixture were still present in equal amounts.

## EXAMPLE 11

Virus generation of an enhanced adenoviral construct – “MRK Ad5 HIV-1gag”

The results obtained from the competition study allowed us to make the following conclusions: (1) The packaging signal extension is beneficial; (2) Presence of E3 does enhance viral growth; (3) E1 parallel orientation is recommended; and (4) PolyA signals have no effect on the growth of the adenovirus.

MRK Ad5 HIV-1 gag exhibited the most desirable results. This construct contains the hCMV(no intron)-FLgag-bGHpA transgene inserted into the new E3+ adenovector backbone, MRKpAdHVE3, in the E1 parallel orientation. We have designated this adenovector MRK Ad5 HIV-1 gag. This construct was prepared as outlined below:

The pre-plasmid MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA was digested with *Pac1* to release the vector backbone and 3.3 µg was transfected by calcium phosphate method (Amersham Pharmacia Biotech.) in a 6 cm dish containing PER.C6<sup>®</sup> cells at ~60% confluence. Once CPE was reached (7-10 days), the culture was freeze/thawed three times and the cell debris pelleted. 1 ml of this cell lysate was used to infect into a 6 cm dish containing PER.C6<sup>®</sup> cells at 80-90% confluence. Once CPE was reached, the culture was freeze/thawed three times and the cell debris pelleted. The cell lysate was then used to infect a 15 cm dish containing PER.C6<sup>®</sup> cells at 80-90% confluence. This infection procedure was continued and expanded at passage 6. The virus was then extracted from the cell pellet by CsCl method. Two bandings were performed (3-gradient CsCl followed by a continuous CsCl gradient). Following the second banding, the virus was dialyzed in A105 buffer. Viral DNA was extracted using pronase treatment followed by phenol chloroform. The viral DNA was then digested with *HindIII* and radioactively labeled with [<sup>33</sup>P]dATP. Following gel electrophoresis to separate the digestion products the gel was dried down on Whatman paper and then subjected to autoradiography. The digestion products were compared with the digestion products from the pre-plasmid (that had been digested with *Pac1/HindIII* prior to labeling). The expected sizes were observed, indicating that the virus had been successfully rescued. This strategy was used to rescue virus from each of the various adenovector plasmid constructs prepared.

## EXAMPLE 12

Stability Analyses

To determine whether the various adenovector constructs (e.g., MRK Ad5 HIV-1 gag) show genetic stability, the viruses were each passaged continually. The viral DNA was analyzed at passages 3, 6 and 10. Each virus maintained its correct genetic structure. In addition, the stability of the MRK Ad5 HIV-1 gag was analyzed under propagation conditions similar to that performed in large scale production. For this analysis, the transfections of MRK Ad5 HIV-1 gag as well as three other adenoviral vectors were repeated and the virus was purified at P3. The three other adenovectors were as follows: (1) that comprising hCMV(no intron)-Flgag with a bGHpA terminator in an E3- adenovector backbone; (2) that comprising hCMV(no intron)-Flgag with a SPA termination signal in an E3+ adenovector backbone, and that comprising a mCMV-Flgag with a bGHpA terminator in an E3+ adenovector backbone. All of the vectors have the transgene inserted in the E1 parallel orientation. Viral DNA was analyzed by radioactive restriction analysis to confirm that it was correct before being delivered to fermentation cell culture for continued passaging in serum-free media. At P5 each of the four viruses were purified and the viral DNA extracted for analysis by the restriction digestion and radiolabeling procedure. This virus has subsequently been used in a series of studies (*in vitro* gag expression in COS cells, rodent study and rhesus monkey study) as will be described below. The viruses from P5 are shown in Figure 9.

The passaging under serum-free conditions was continued for the MRKHVE3 (transgene-less, obtained from MRKpAdHVE3 pre-plasmid) and the MRKAd5HIV-1gag (obtained from MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA pre-plasmid) viruses. Figure 10 shows viral DNA analysis by radioactive restriction digestion at passage 11 for MRKHVE3, MRKAd5HIV-1gagE3-, and passage 11 and 12 for MRKAd5HIV-1gag. Aside from the first lane which is the DNA marker lane, the next three lanes are virus from the pre-plasmid controls (controls based on the original virus) - MRKpAdHVE3 (also referred to as "pMRKHVE3"), MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA, and pMRKAd5gag(E3-), respectively. As seen in Figure 10, each of the viral DNA samples show the expected bands with no extraneous bands showing. This signifies that there are no major variant adenovirus species present that can be detected by autoradiography.

Figure 11 shows the results of viral competition study between MRKHVE3 and MRKAd5HIV-1gag. These viruses were mixed together at equal MOI (140 viral

particles each; 280 vp total) at passage 6 and continued to be passaged until P11. Aside from the first lane which is the DNA marker lane, the next two lanes are the pre-plasmid controls obtained from MRKpAdHVE3 and MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA. The next two lanes are the viral DNA from the starting viral material at passage six. The last two lanes are the competition studies performed in duplicate. The data in Figure 11 shows the effect the gag transgene in culture. Growth of a MRKAd5gag virus was compared with growth of a "transgene-less" MRKHVE3. These two viruses were infected at the same MOI (i.e. 140 vp each) at passage 6 and then passaged through to passage 11 and the viral pool was analyzed by radioactive restriction analysis. The data shows that one virus did not out compete the other. Therefore, the gag transgene did not show obvious signs of toxicity to the adenovirus.

Analysis by *HindIII* digestion shows that each virus specie is present in approximately equal amounts. As above, there does not appear to be signs of any extraneous bands. Figure 12 shows higher passage numbers for MRKAd5HIV-1gag grown under serum-containing conditions. The genome integrity again has been maintained and there is no evidence of rearrangements, even at the highest passage level (P21).

Each of the four vectors shown in Figure 9 were analyzed for amplification capacity. Table 4 below shows the QPA analysis used in the estimation of viral amplification ratios at P4. The determination of the amplification ratio for the original HIV-1 gag construct is based on the clinical lot at P12. It has been shown that amplification rates increases with higher passage number for the original virus. The reason for this observation is due to the emergence of variants which exhibit increased growth rates compared to the intact adenovector. With continued passaging of the original Ad gag vector, the level of variants increases and hence amplification rates increase also.

The MRK Ad5 HIV-1 gag virus has also been continually passaged under process conditions (i.e., serum-free media). Viral DNA extracted from passages 11 and 12 show no evidence of rearrangement.

Table 4:  
Amplification Ratios Based on AEX and QPA Analysis of  
Virus Amplification from Passage 3 to Passage 4.

Ad gag construct	Amplification Ratio
MRKAd5 <sub>gag</sub>	470
HCMV-Flgag-bGHpA [E3-]	115
HCMV-Flgag-SPA [E3+]	320
mCMV-FLgag-bGHpA [E3+]	420
Original construct *	40 - 50

\* This estimation is based on the clinical lot growth characteristics at Passage 12.

### EXAMPLE 13

#### Analytical Evaluation of the enhanced Ad5 Constructs

To study the effects of the transgene and the E3 gene on virus amplification, the enhanced adenoviral vector, MRK Ad5 HIV-1 gag, along with its transgene-less version (MRKpAdHVE3) and its E3- version (MRK Ad5 HIV-1 gag E3-), was studied for several passages under serum-free conditions. Table 5A shows the amplification ratios determined for passages P3 to P8 for MRK Ad5 HIV-1 gag. Within a certain MOI range, it has been determined that the virus output is directly proportional to the virus input. Therefore, the greater the number of virus particles per cell at infection, the greater the virus amount produced. Viral amplification ratios, on the other hand, are inversely proportional to the virus input. The lower the virus input, the greater the amplification ratio.

Table 5B shows the amplification rates of the new E3+ vector backbone MRKpAdHVE3. It has a significantly lower rate of amplification compared with the gag transgene containing version. This may be contributed to the larger size MRK Ad5 HIV-1 gag since it contains the transgene. This inclusion of the transgene brings the size of the adenovirus closer to the size of a wild type Ad5 virus. It is well known that adenoviruses amplify best when they are at close to their wild type genomic size.



Wild type Ad5 is 35,935 bp. The MRKpAdHVE3 is 32, 905 bp in length. The enhanced adenovector MRK Ad5 HIV-1 gag is 35,453bp (See Figure 14 for vector map; see also Figure 15A-X show the complete pre-adenoviral vector sequence, which includes an additional 2,021 bp of the vector backbone).

- 5           Table 5C shows the amplification rates of the new E3- gag containing virus MRK Ad5 HIV-1 gag E3-. Once again, this virus shows lower growth rate than the enhanced adenoviral vector. This may be attributed to the decreased sized of this virus (due to the E3 gene deletion) compared with wild type Ad5. The MRK Ad5 HIV-1 gag E3- virus is 32,810 bp in length. This can be compared with the wild type
- 10 Ad5 which is 35,935 bp and MRK Ad5 HIV-1 gag which is 35,453 bp in length.

**Table 5A:** Amplification ratios determined by AEX and QPA for **MRKAd5gag** over several continuous passaging in serum free media. Following P5, two replicate samples were taken (rep-1 and rep-2) and analyzed.

### *MRKAd5gag rep1*

	Xv (10 <sup>6</sup> cells/ml) Infection	Viability (%) Harvest	Harvest Time h.p.i.	Cell Passage Number	Titer 10 <sup>6</sup> vp/ml culture	Titer 10 <sup>4</sup> vp/cell	QPA 10 <sup>6</sup> TCID <sub>50</sub> /ml	Ratio AEX:QPA	Amplification Ratio	AEX Internal Control
P4	1.49, 81%	0.58, 50%	44	46	6.7	5.9	1.72	50	470 (MOI = 125)	
P5	1.38, 83%	0.56, 47%	48	49	6.7	4.9	1.38	49	170	
P6	1.04, 84%	0.68, 77%	47	48	5.8	5.6	1.42	41	200	
P7	1.50, 84%	0.86, 81%	49.5	50	3.9	1.4	0.87	40	50	
P7	1.09, 97%	0.78, 59%	50	52	5.2	4.7	1.70	31	170	
P8	1.03, 84%	0.88, 84%	47.5	54	9.0	8.7	1.10	82	310	
P9	0.89, 85%	0.99, 73%	47.5	56	4.4	4.9	1.03	43	175	3.12 2.64
P10	1.09, 81%	1.06, 66%	47.5	58	3.0	2.8	1.16	26	100	2.70 2.60
P11	1.18, 88%	0.98, 65%	47	60	3.8	3.0	1.15	31	110	2.70 2.70
P12	0.98, 91%	0.85, 63%	47.5	47	5.4	5.5	1.20	45	200	2.88 2.60
P13	1.00, 89%	0.70, 67%	49	49	5.8	5.8	1.11	62	210	3.18 3.18
P14	1.94, 92%	0.88, 67%	48	53	8.8	4.4			160	3.28 3.27
P15	0.97, 98%	0.84, 66%	47	47	6.9	7.1			250	3.12 2.91

**Table 5B:** Amplification ratios determined by AEX and QPA for **MRKHVE3** over several continuous passaging in serum free media. **MRKHVE3** is the new vector backbone which does NOT carry a transgene.

### *MRKHVE3*

	Xv (10 <sup>6</sup> cells/ml) Infection	Viability (%) Harvest	Harvest Time h.p.i.	Cell Passage Number	Titer 10 <sup>6</sup> vp/ml culture	Titer 10 <sup>4</sup> vp/cell	QPA 10 <sup>6</sup> TCID <sub>50</sub> /ml	Ratio AEX:QPA	Amplification Ratio	AEX Internal Control
P4	1.10, 97%	1.28, 76%	49	54	4.1	3.8	1.70	23	300 (MOI = 125)	
P5	0.92, 89%	1.18, 77%	47	48	4.3	4.7	1.24	35	170	
P6	1.53, 88%	1.28, 76%	49.5	50	1.2	0.8	0.56	21	30	
P6	1.09, 97%	1.11, 81%	49	52	4.0	3.6	1.18	34	130	
P7	1.17, 91%	1.22, 91%	47.5	54	3.7	3.2	0.50	74	110	
P8	0.98, 88%	1.41, 63%	48	56	2.1	2.1	0.47	46	75	3.12 2.64
P9	1.20, 89%	1.28, 81%	47.5	58	0.8	0.7	0.29	28	25	2.70 2.60
P10	0.99, 82%	1.53, 86%	47	60	2.3	2.3	0.43	53	80	2.70 2.70
P11	1.07, 96%	1.25, 83%	48	47	2.7	2.5	0.41	66	90	2.86 2.80
P12	0.80, 91%	1.14, 80%	49.5	49	5.9	7.4	0.48	123	250	3.18 3.18
P13	1.98, 95%	1.14, 85%	45.5	53	5.8	3.0			110	3.28 3.27
P14	0.97, 96%	1.03, 98%	48.5	47	9.4	9.7			850	3.12 2.91
P15	0.87, 99%	0.97, 59%	49.5	49	5.3	6.1			218	2.78 2.52

Table 5C. Amplification ratios determined by AEX and QPA for MRKAd5gag(E3-) over several continuous passaging in serum free media. This construct is identical to the MRKAd5gag construct except that this version is DELETED of the E3 gene.

5

### MRKAd5gag(E3-)

	Xv (10 <sup>6</sup> osts/ml), Infection	Viability (%), Harvest	Harvest Time h.p.i.	Cell Passage Number	Titer 10 <sup>6</sup> vp/ml culture	Titer 10 <sup>6</sup> vp/cell	QPA 10 <sup>6</sup> TCID <sub>50</sub> /ml	Ratio AEX:QPA	Amplification Ratio	AEX Internal Control
P4	1.62, 77%	1.12, 82%	47.5	46	2.0	1.2	0.92	20	100 (MOI=125)	
P5	1.16, 82%	0.62, 43%	49	49	3.3	2.9	0.99	34	100	
P6	1.71, 86%	0.20, 10%	49	50	4.7	2.7	1.70	28	100	
P6	1.09, 87%	0.63, 54%	49.5	52	5.4	5.0	1.76	31	180	
P7	1.17, 91%	0.88, 72%	47.50	54	7.1	6.1	0.67	106	220	
P8	0.98, 88%	0.77, 48%	48	56	3.1	3.2	0.66	47	115	3.12 2.84
P9	1.20, 89%	1.03, 72%	48	58	1.8	1.6	0.57	32	55	2.70 2.60
P10	0.99, 82%	0.80, 62%	46.5	60	3.2	3.2	0.68	47	115	2.70 2.70
P11	1.07, 95%	0.98, 70%	48.5	47	5.9	5.5	0.68	87	200	2.86 2.60
P12	0.80, 91%	0.67, 59%	50	49	5.1	6.4	0.72	71	230	3.18 3.18
P13	1.98, 95%	0.91, 59%	45.5	53	7.4	3.8			135	3.28 3.27
P14	0.97, 95%	0.81, 74%	48	47	6.8	7.0			250	3.12 2.91
P15	0.87, 99%	0.84, 56%	49	49	4.8	5.5			198	2.78 2.52

### EXAMPLE 14

#### Gag Expression Analysis of the Novel Constructs

*In vitro* gag analysis of the MRK Ad5 HIV-1 gag and the original HIV-gag vectors (research and clinical lot) show comparable gag expression. The clinical lot shows only a slightly reduced gag expression level. The most noticeable difference is with the mCMV vector. This vector shows roughly 3 fold lower expression levels compared with the other vectors tested (which all contain hCMV promoters). The mCMV-FLgag with bGHpA assay was performed three times using different propagation and purification lots and it consistently exhibited weaker gag expression.

### EXAMPLE 15

#### Evaluation of MRK Ad5 HIV-1 gag and Other gag-Containing Adenovectors in Balb/c Mice

Cohorts of 10 balb/c mice were vaccinated intramuscularly with escalating doses of MRK Ad5 HIV-1 gag, and the research and clinical lots of original Ad5HIV-1gag. Serum samples were collected 3 weeks post dose 1 and analyzed by anti-p24 sandwich ELISA.

Anti-p24 titers in mice that received MRK Ad5 HIV-1 gag ( $10^7$  and  $10^9$  vp(viral particle) doses) were comparable (Figure 13) to those of the research lot of Ad5HIV-1 gag, for which much of the early rhesus data were generated on. These titers were also comparable when E3 is deleted (MRKAd5hCMVgagbGHpA(E3-)) or SPA is substituted for bGHpA terminator (MRKAd5 hCMV-gag-SPA (E3+)) or murine CMV promoter is used in place of hCMV (MRKAd5 mCMV-gag-bGHpA (E3+)) in the MRKAd5 backbone.

The results shown in Table 7 indicate that the three other vectors ( in addition to the preferred vector, MRK Ad5 HIV-1 gag, are also capable of inducing strong anti-gag antibody responses in mice. Interestingly enough, while the mCMV-FLgag construct containing bGHpA and E3+ in an E1 parallel orientation showed lowest gag expression in the COS cell *in vitro* infection (Table 6) in comparison with the other vectors tested, it generated the greatest anti-gag antibody response this *in vivo* Balb/c study. Table 7 also shows a dose response in anti-gag antibody production in both the research and the clinical lot. As expected, the clinical lot shows reduced anti-gag antibody induction at each dosage level compared to the same dosage used for the research lot.

Table 6: *In vitro* analysis for gag expression in COS cells by Elisa assay.

Viral Vectors <sup>a</sup>	$\mu\text{g gag}/4.8 \times 10^5 \text{ COS}/10^8 \text{ parts}/48\text{hr}$
MRKAd5gag <sup>b</sup>	1.40
Clinical lot Ad5gag <sup>c</sup>	1.28
Research lot Ad5gag <sup>d</sup>	1.32
MCMVFL-gagbGHpA <sup>e</sup>	0.42

<sup>a</sup>  $A_{260\text{nm}}$  absorbance readings taken for viral particle determinations.

<sup>b</sup> MRKAd5gag was produced in serum free conditions and purified at P5.

<sup>c</sup> Clinical lot# Ad5gagFN0001

<sup>d</sup> Research Ad5FLgag lot# 6399

<sup>e</sup> mCMVFL-gagbGHpA was produced in serum free conditions and purified at P5.

**Table 7:** mHIV020 Anti-p24 Ab Titers in Balb/c mice (n=10) vaccinated with various Adgag constructs and lots (3 week post dose1).

Group ID	Vaccine	Dose (vp)	GMT	SE upper	SE lower
1	<sup>a</sup> MRKAd5gag	10 <sup>7</sup>	25600	5877	4780
2	"	10 <sup>9</sup>	409600	94028	76473
3	hCMV FL-gag bGHpA [E3-] →	10 <sup>7</sup>	7352	2077	1620
4	"	10 <sup>9</sup>	235253	59767	47659
5	hCMV FL-gag SPA [E3+] →	10 <sup>7</sup>	12800	9905	236
6	"	10 <sup>9</sup>	310419	99181	75165
7	<sup>b</sup> mCMV FL-gag bGHpA [E3+] →	10 <sup>7</sup>	44572	23504	15389
8	"	10 <sup>9</sup>	941014	239068	190636
9	<sup>c</sup> hCMV FL-gag bGHpA [E3-] ←	10 <sup>7</sup>	3676	934	745
10	"	10 <sup>9</sup>	117627	17491	15227
11	research lot hCMV IntronA FL-gag bGHpA [E3-] <-	10 <sup>6</sup>	528	262	175
12	"	10 <sup>7</sup>	14703	5274	3882
13	"	10 <sup>8</sup>	58813	14942	11915
14	"	10 <sup>9</sup>	204800	53232	42250
15	clinical lot hCMVintronA FL-gag bGHpA [E3-] <-	10 <sup>6</sup>	230	82	61
16	"	10 <sup>7</sup>	4222	3405	1138
17	"	10 <sup>8</sup>	19401	3939	3274
18	"	10 <sup>9</sup>	89144	25187	19639
19	Naïve	none	93	7	6

\*2x50 µL i.m. (quad) injections/animal

P.I.s: Youil, Chen, Casimiro

Vaccination: T. Toner, Q. Su

Assay: M. Chen

<sup>a</sup>The structure of MRKAd5gag is: hCMVFL-gagbGHpA [E3+] → The same lot of MRKAd5gag used in this rodent study was used in the Rhesus monkey study (Tables 7 and 8).

<sup>b</sup>The same lot of mCMVFL-gagbGHpA[E3+] used in the *in vitro* study (Table 6) was used here.

<sup>c</sup>This construct was designed by Volker Sandig. It contains a shorter version of the hCMV promoter than that used in the MRK constructs. The adenovector backbone is identical to the original backbone used in the original Adgag vector. Expression at 10<sup>6</sup>7 dose from this vector is 7 fold lower than the same dose of the MRKAd5gag and 4 fold lower than the research lot.

## EXAMPLE 16

### Comparison of Humoral and Cellular Responses Towards the Original Ad-gag Construct with the New MRK Ad5 HIV-1 gag in Rhesus Monkeys

- 5 Cohorts of 3 rhesus monkeys were vaccinated intramuscularly with MRK Ad5 HIV-1 gag or the clinical Ad5gag bulk at two doses, 10<sup>11</sup> vp and 10<sup>9</sup> vp. Immunizations were conducted at week 0, 4, and 25. Serum and PBMC samples were collected at selected time points. The serum sample were assayed for anti-p24 Ab titers (using competitive based assay) and the PBMCs for antigen-specific IFN-  
10 gamma secretion following overnight stimulation with gag 20-mer peptide pool (via ELISpot assay).

The results shown in Table 8 indicate comparable responses with respect to the generation of anti-gag antibodies. The frequencies of gag-specific T cells in

- peripheral blood assmumarized in Table 9 demonstrate a strong cellular immune response generated after a single dose with the new construct MRK Ad5 HIV-1 gag. The responses are also boostable with second dose of the same vector. The vector is also able to induce CD8+ T cell responses (as evident by remaining spot counts after CD4+ depletion of PBMCs) which are responsible for cytotoxic activity.
- 5

Table 8 Anti-p24 antibody titers (in mMU/mL) in rhesus macaques immunized with gag-expressing adenovectors (Protocol HIV203).

Vaccine	Pre	Wk 4	Wk 8	Wk 12	Wk 16	Wk 20	Wk 25	Wk 28
MRKAd5gag <sup>P</sup> , 10 <sup>11</sup> vp								
97N010	<10	118	5528	11523	7062	21997	ND	51593
97N116	<10	62	772	1447	1562	2174	ND	20029
98X007	<10	66	3353	6156	6845	3719	ND	24031
MRKAd5gag, 10 <sup>9</sup> vp								
97N120	<10	51	204	318	366	482	ND	6550
97N144	<10	18	118	274	706	888	ND	7136
98X008	<10	15	444	386	996	1072	ND	12851
Ad5gag <sup>P</sup> , Clinical Lot, 10 <sup>11</sup> vp								
97X001	<10	87	2579	4718	7174	7250	ND	69226
97N146	<10	72	3604	7380	7526	18906	ND	60283
98X009	<10	78	4183	3946	3124	6956	ND	26226
Ad5gag, Clinical Lot, 10 <sup>9</sup> vp								
97N020	<10	<10	143	371	390	1821	ND	17177
97X003	<10	<10	39	93	156	596	ND	2053
98X012	<10	81	342	717	956	1558	ND	11861
<sup>a</sup> MRKAd5gag (hCMV, bGHpA, E3+)								
<sup>b</sup> original Ad5gag vector (hCMV/Intron A, bGHpA, E3-), lot#FN0001								
ND, not determined								

10

Table 9. Number of gag-specific T cells per million peripheral blood mononuclear cells (PBMCs) in rhesus monkeys immunized with gag-expressing adenovectors. Also included are those frequencies in PBMCs depleted of CD4<sup>+</sup> T cells.

Grp #	Vaccination T=0,4,25 wks	Monkey ID	T=4 Wk		T=6 Wk		T=11 Wk		T=16 Wk		T=25 Wk		T=28 Wk	
			Media <sup>a</sup>	Gag H <sup>b</sup>	Media	Gag H	Media	Gag H	Media	Gag H	Media	Gag H	Media	Gag H
1	MRKAd5gag 10 <sup>8</sup> vp	97N010	8	89	0	395	0	1058	0	1174	3	775	4	1074
		97N010(CD4-)	4	38			3	993			0	76	0	594
		97N116	1	398	1	609	0	534	4	395	1	261	0	408
		97N116(CD4-)	11	676			0	593			0	184	0	666
		98X007	10	578	0	1304	3	2193	1	2118	3	1588	0	2113
		98X007(CD4-)	20	965			0	2675			0	1656	0	1278
2	MRKAd5gag 10 <sup>9</sup> vp	97N120	5	275	1	249	4	141	4	119	9	206	4	219
		97N120(CD4-)	11	170			0	85			0	75	1	219
		97N144	3	236	6	438	1	318	3	256	1	98	5	373
		97N144(CD4-)	6	148			0	285			ND	0	0	625
		98X008	4	368	1	1090	3	891	4	673	3	473	5	735
		98X008(CD4-)	14	696			0	1175			0	391	4	848
3	Ad5gag clinical lot 10 <sup>8</sup> vp	97X001	0	281	1	485	0	817	0	1220b	1	894	0	1858
		97X001(CD4-)	10	283			3	996			0	1010	0	1123
		97N146	3	150	1	485	0	339	1	1272	3	1238	3	1785
		97N146(CD4-)	6	133			0	370			0	654	0	971
		98X009	0	93	3	339	3	559	0	896	1	384	0	1748
		98X009(CD4-)	0	73			0	333			0	225	0	644
4	Ad5gag clinical lot 10 <sup>9</sup> vp	97N020	3	30	1	101	0	66	0	36	0	26	0	41
		97N020(CD4-)	10	29			0	15			0	1	0	16
		97X003	4	68	5	134	0	18	1	38	4	38	6	81
		97X003(CD4-)	9	40			0	6			0	4	0	19
		98X012	5	95	3	54	1	34	0	18	0	20	1	121
		98X012(CD4-)	11	70			0	11			0	8	0	41
5	Naïve	96R041	6	8	1	1	0	0	0	0	0	0	1	0
		053F	14	18	5	16	20	14	19	15	10	15	24	9

Based on either 4x10<sup>6</sup> or 2x10<sup>6</sup> cells per well (depending on spot density)

ND, not determined

<sup>a</sup>Track or no peptide control

<sup>b</sup>Pool of 20-aa peptides overlapping by 10 aa and encompassing the gag sequence

5

The adenovectors described herein and, particularly, MRK Ad5 HIV-1 gag, represent very promising HIV-gag adenovectors with respect to their enhanced growth characteristics in both serum and, more importantly, in serum-free media conditions. In comparison with the current HIV-1 gag adenovector construct, MRK Ad5 HIV-1 gag shows a 5-10 fold increased amplification rate. We have shown that it is genetically stable at passage 21. This construct is able to generate significant cellular immune responses *in vivo* even at a relatively low dose of 10<sup>8</sup> vp. The potency of the MRKAd5gag construct is comparable to, if not better than the original HIV-1gag vector as shown in this rhesus monkey study.

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#### EXAMPLE 17

#### CODON OPTIMIZED HIV-1 POL AND CODON OPTIMIZED HIV-1 POL MODIFICATIONS

20

The open reading frames for the various synthetic *pol* genes disclosed herein comprise coding sequences for the reverse transcriptase (or RT which consists of a polymerase and RNase H activity) and integrase (IN). The protein sequence is based

on that of Hxb2r, a clonal isolate of IIB; this sequence has been shown to be closest to the consensus clade B sequence with only 16 nonidentical residues out of 848 (Korber, et al., 1998, Human retroviruses and AIDS, Los Alamos National Laboratory, Los Alamos, New Mexico). The skilled artisan will understand after  
 5 review of this specification that any available HIV-1 or HIV-2 strain provides a potential template for the generation of HIV pol DNA vaccine constructs disclosed herein. It is further noted that the protease gene is excluded from the DNA vaccine constructs of the present invention to insure safety from any residual protease activity in spite of mutational inactivation. The design of the gene sequences for both wild-  
 10 type (wt-pol) and inactivated pol (IA-pol) incorporates the use of human preferred ("humanized") codons for each amino acid residue in the sequence in order to maximize *in vivo* mammalian expression (Lathe, 1985, *J. Mol. Biol.* 183:1-12). As can be discerned by inspecting the codon usage in SEQ ID NOs: 1, 3, 5 and 7, the following codon usage for mammalian optimization is preferred: Met (ATG), Gly  
 15 (GGC), Lys (AAG), Trp (TGG), Ser (TCC), Arg (AGG), Val (GTG), Pro (CCC), Thr (ACC), Glu (GAG); Leu (CTG), His (CAC), Ile (ATC), Asn (AAC), Cys (TGC), Ala (GCC), Gln (CAG), Phe (TTC) and Tyr (TAC). For an additional discussion relating to mammalian (human) codon optimization, see WO 97/31115 (PCT/US97/02294), which, as noted elsewhere in this specification, is hereby incorporated by reference. It  
 20 is intended that the skilled artisan may use alternative versions of codon optimization or may omit this step when generating HIV pol vaccine constructs within the scope of the present invention. Therefore, the present invention also relates to non-codon optimized versions of DNA molecules and associated recombinant adenoviral HIV vaccines which encode the various wild type and modified forms of the HIV Pol  
 25 protein disclosed herein. However, codon optimization of these constructs is a preferred embodiment of this invention.

A particular embodiment of this portion of the invention comprises codon optimized nucleotide sequences which encode wt-pol DNA constructs (herein, "wt-pol" or "wt-pol (codon optimized)") wherein DNA sequences encoding the protease  
 30 (PR) activity are deleted, leaving codon optimized "wild type" sequences which encode RT (reverse transcriptase and RNase H activity) and IN integrase activity. A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:1, the open reading frame being contained from an initiating Met residue at nucleotides 10-12 to a termination codon from nucleotides 2560-2562. SEQ ID NO:1 is as follows:

35 AGATCTACCA TGGCCCCCAT CTCCCCATT GAGACTGTGC CTGTGAAGCT GAAGCCTGGC  
 ATGGATGGCC CCAAGGTGAA GCAGTGGCCC CTGACTGAGG AGAAGATCAA GGCCCTGGTG



GAAATCTGCA CTGAGATGGA GAAGGAGGGC AAAATCTCCA AGATTGGCCC CGAGAACCCC  
 TACAACACCC CTGTGTTTGC CATCAAGAAG AAGGACTCCA CCAAGTGGAG GAAGCTGGTG  
 GACTTCAGGG AGCTGAACAA GAGGACCCAG GACTTCTGGG AGGTGCAGCT GGGCATCCCC  
 CACCCCGCTG GCCTGAAGAA GAAGAAGTCT GTGACTGTGC TGGATGTGGG GGATGCCTAC  
 5 TTCTCTGTGC CCCTGGATGA GGAATTTCAGG AAGTACACTG CCTTCACCAT CCCCTCCATC  
 AACAAATGAGA CCCCTGGCAT CAGGTACCAG TACAATGTGC TGCCCCAGGG CTGGAAGGGC  
 TCCCCTGCCA TCTTCAGTC CTCCATGACC AAGATCCTGG AGCCCTTCAG GAAGCAGAAC  
 CCTGACATTG TGATCTACCA GTACATGGAT GACCTGTATG TGGGCTCTGA CCTGGAGATT  
 GGGCAGCACA GGACCAAGAT TGAGGAGCTG AGGCAGCACC TGCTGAGGTG GGGCCTGACC  
 10 ACCCTTGACA AGAAGCACCA GAAGGAGCCC CCCTTCCTGT GGATGGGCTA TGAGCTGCAC  
 CCCGACAAGT GGAATGTGCA GCCCATTTGTG CTGCCCTGAGA AGGACTCCTG GACTGTGAAT  
 GACATCCAGA AGCTGGTGGG CAAGCTGAAC TGGGCCCTCC AAATCTACCC TGGCATCAAG  
 GTGAGGCAGC TGTGCAAGCT GCTGAGGGGC ACCAAGGCCC TGAAGAGGT GATCCCCCTG  
 ACTGAGGAGG CTGAGCTGGA GCTGGCTGAG AACAGGGAGA TCCTGAAGGA GCCTGTGCAT  
 15 GGGGTGTACT ATGACCCCTC CAAGGACCTG ATTGCTGAGA TCCAGAAGCA GGGCCAGGGC  
 CAGTGGACCT ACCAAATCTA CCAGGAGCCC TTCAAGAACC TGAAGACTGG CAAGTATGCC  
 AGGATGAGGG GGGCCACAC CAATGATGTG AAGCAGCTGA CTGAGGCTGT GCAGAAGATC  
 ACCACTGAGT CCATTGTGAT CTGGGGCAAG ACCCCCAAGT TCAAGCTGCC CATCCAGAAG  
 GAGACCTGGG AGACCTGGTG GACTGAGTAC TGGCAGGCCA CCTGGATCCC TGAGTGGGAG  
 20 TTTGTGAACA CCCCCCCCCT GGTGAAGCTG TGGTACCAGC TGGAGAAGGA GCCCATTGTG  
 GGGGCTGAGA CCTTCTATGT GGATGGGGCT GCCAACAGGG AGACCAAGCT GGGCAAGGCT  
 GGCTATGTGA CCAACAGGGG CAGGCAGAAG GTGGTGACCC TGAAGTACAC CACCAACCAG  
 AAGACTGAGC TCCAGGCCAT CTACCTGGCC CTCCAGGACT CTGGCCTGGA GGTGAACATT  
 GTGACTGACT CCCAGTATGC CCTGGGCATC ATCCAGGCCC AGCCTGATCA GTCTGAGTCT  
 25 GAGCTGGTGA ACCAGATCAT TGAGCAGCTG ATCAAGAAGG AGAAGGTGTA CCTGGCCTGG  
 GTGCCTGCCC ACAAGGGCAT TGGGGGCAAT GAGCAGGTGG ACAAGCTGGT GTCTGCTGGC  
 ATCAGGAAGG TGCTGTTTCT GGATGGCATT GACAAGGCCC AGGATGAGCA TGAGAAGTAC  
 CACTCCAACCT GGAGGGCTAT GGCCTCTGAC TTCAACCTGC CCCCTGTGGT GGCTAAGGAG  
 ATTGTGGCCT CCTGTGACAA GTGCCAGCTG AAGGGGGAGG CCATGCATGG GCAGGTGGAC  
 30 TGCTCCCCCTG GCATCTGGCA GCTGGACTGC ACCCACCTGG AGGGCAAGGT GATCCTGGTG  
 GCTGTGCATG TGGCCTECGG CTACATTGAG GCTGAGGTGA TCCCTGCTGA GACAGGCCAG  
 GAGACTGCCT ACTTCCTGCT GAAGCTGGCT GGCAGGTGGC CTGTGAAGAC CATCCACACT  
 GACAATGGCT CCAACTTCAC TGGGGCCACA GTGAGGGCTG CCTGCTGGTG GGCTGGCATC  
 AAGCAGGAGT TTGGCATCCC CTACAACCCC CAGTCCCAGG GGGTGGTGGG GTCCATGAAC  
 35 AAGGAGCTGA AGAAGATCAT TGGGCAGGTG AGGGACCAGG CTGAGCACCT GAAGACAGCT  
 GTGCAGATGG CTGTGTTTCAT CCACAACCTC AAGAGGAAGG GGGGCATCGG GGGCTACTCC

GCTGGGGAGA GGATTGTGGA CATCATTGCC ACAGACATCC AGACCAAGGA GCTCCAGAAG  
 CAGATCACCA AGATCCAGAA CTTCAAGGGTG TACTACAGGG ACTCCAGGAA CCCCCTGTGG  
 AAGGGCCCTG CCAAGCTGCT GTGGAAGGGG GAGGGGGCTG TGGTGATCCA GGACAACTCT  
 GACATCAAGG TGGTGCCCAG GAGGAAGGCC AAGATCATCA GGGACTATGG CAAGCAGATG  
 5 GCTGGGGATG ACTGTGTGGC CTCCAGGCAG GATGAGGACT AAAGCCCGGG CAGATCT (SEQ  
 ID NO:1).

The open reading frame of the wild type pol construct disclosed as SEQ ID  
 NO:1 contains 850 amino acids, disclosed herein as SEQ ID NO:2, as follows:

Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro  
 10 Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys  
 Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys  
 Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala  
 Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg  
 Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile  
 15 Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Asp  
 Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys  
 Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile  
 Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala  
 Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln  
 20 Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Asp Asp Leu Tyr Val Gly  
 Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg  
 Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln  
 Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys  
 Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val  
 25 Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile  
 Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr  
 Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu  
 Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr  
 Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln  
 30 Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys  
 Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys  
 Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile  
 Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp  
 Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp  
 35 Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu  
 Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Asp Gly Ala Ala

Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly  
 Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Glu  
 Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn  
 Ile Val Thr Asp Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro  
 5 Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile  
 Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile  
 Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys  
 Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys  
 Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro  
 10 Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys  
 Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln  
 Leu Asp Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His  
 Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly  
 Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val  
 15 Lys Thr Ile His Thr Asp Asn Gly Ser Asn Phe Thr Gly Ala Thr Val  
 Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro  
 Tyr Asn Pro Gln Ser Gln Gly Val Val Glu Ser Met Asn Lys Glu Leu  
 Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr  
 Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly  
 20 Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr  
 Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn  
 Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro  
 Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn  
 Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp  
 25 Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp  
 Glu Asp (SEQ ID NO:2).

The present invention especially relates to an adenoviral vector vaccine which  
 comprises a codon optimized HIV-1 DNA pol construct wherein, in addition to  
 deletion of the portion of the wild type sequence encoding the protease activity, a  
 30 combination of active site residue mutations are introduced which are deleterious to  
 HIV-1 pol (RT-RH-IN) activity of the expressed protein. Therefore, the present  
 invention preferably relates to an adenoviral HIV-1 DNA pol-based vaccine wherein  
 the construct is devoid of DNA sequences encoding any PR activity, as well as  
 containing a mutation(s) which at least partially, and preferably substantially,  
 35 abolishes RT, RNase and/or IN activity. One type of HIV-1 pol mutant which is part  
 and parcel of an adenoviral vector vaccine may include but is not limited to a mutated

DNA molecule comprising at least one nucleotide substitution which results in a point mutation which effectively alters an active site within the RT, RNase and/or IN regions of the expressed protein, resulting in at least substantially decreased enzymatic activity for the RT, RNase H and/or IN functions of HIV-1 Pol. In a preferred embodiment of this portion of the invention, a HIV-1 DNA pol construct contains a mutation or mutations within the Pol coding region which effectively abolishes RT, RNase H and IN activity. An especially preferable HIV-1 DNA pol construct in a DNA molecule which contains at least one point mutation which alters the active site of the RT, RNase H and IN domains of Pol, such that each activity is at least substantially abolished. Such a HIV-1 Pol mutant will most likely comprise at least one point mutation in or around each catalytic domain responsible for RT, RNase H and IN activity, respectfully. To this end, an especially preferred HIV-1 DNA pol construct is exemplified herein and contains nine codon substitution mutations which results in an inactivated Pol protein (IA Pol: SEQ ID NO:4, Figure 17A-C) which has no PR, RT, RNase or IN activity, wherein three such point mutations reside within each of the RT, RNase and IN catalytic domains. Therefore, an especially preferred exemplification is an adenoviral vaccine which comprises, in an appropriate fashion, a DNA molecule which encodes IA-pol, which contains all nine mutations as shown below in Table 1. An additional preferred amino acid residue for substitution is Asp551, localized within the RNase domain of Pol. Any combination of the mutations disclosed herein may suitable and therefore may be utilized as an IA-Pol-based vaccine of the present invention. While addition and deletion mutations are contemplated and within the scope of the invention, the preferred mutation is a point mutation resulting in a substitution of the wild type amino acid with an alternative amino acid residue.

Table 1

	<u>wt aa</u>	<u>aa residue</u>	<u>mutant aa</u>	<u>enzyme function</u>
	Asp	112	Ala	RT
	Asp	187	Ala	RT
30	Asp	188	Ala	RT
	Asp	445	Ala	RNase H
	Glu	480	Ala	RNase H
	Asp	500	Ala	RNase H
	Asp	626	Ala	IN
35	Asp	678	Ala	IN
	Glu	714	Ala	IN

It is preferred that point mutations be incorporated into the IApol mutant adenoviral vaccines of the present invention so as to lessen the possibility of altering epitopes in and around the active site(s) of HIV-1 Pol.

To this end, SEQ ID NO:3 discloses the nucleotide sequence which codes for a codon optimized pol in addition to the nine mutations shown in Table 1, disclosed as follows, and referred to herein as "IApol":

```
AGATCTACCA TGGCCCCCAT CTCCCCCATT GAGACTGTGC CTGTGAAGCT GAAGCCTGGC
ATGGATGGCC CCAAGGTGAA GCAGTGGCCC CTGACTGAGG AGAAGATCAA GGCCCTGGTG
GAAATCTGCA CTGAGATGGA GAAGGAGGGC AAAATCTCCA AGATTGGCCC CGAGAACCCC
10 TACAACACCC CTGTGTTTGC CATCAAGAAG AAGGACTCCA CCAAGTGGAG GAAGCTGGTG
GACTTCAGGG AGCTGAACAA GAGGACCCAG GACTTCTGGG AGGTGCAGCT GGGCATCCCC
CACCCCGCTG GCCTGAAGAA GAAGAAGTCT GTGACTGTGC TGGCTGTGGG GGATGCCTAC
TTCTCTGTGC CCCTGGATGA GGACTTCAGG AAGTACACTG CCTTCACCAT CCCCTCCATC
AACAATGAGA CCCCTGGCAT CAGGTACCAG TACAATGTGC TGCCCCAGGG CTGGAAGGGC
15 TCCCCTGCCA TCTTCCAGTC CTCCATGACC AAGATCCTGG AGCCCTTCAG GAAGCAGAAC
CCTGACATTG TGATCTACCA GTACATGGCT GCCCTGTATG TGGGCTCTGA CCTGGAGATT
GGGCAGCACA GGACCAAGAT TGAGGAGCTG AGGCAGCACC TGCTGAGGTG GGGCCTGACC
ACCCCTGACA AGAAGCACCA GAAGGAGCCC CCCTTCCTGT GGATGGGCTA TGAGCTGCAC
CCGACAAGT GGACTGTGCA GCCCATTTGT CTGCCTGAGA AGGACTCCTG GACTGTGAAT
20 GACATCCAGA AGCTGGTGGG CAAGCTGAAC TGGGCCTCCC AAATCTACCC TGGCATCAAG
GTGAGGCAGC TGTGCAAGCT GCTGAGGGGC ACCAAGGCCC TGACTGAGGT GATCCCCCTG
ACTGAGGAGG CTGAGCTGGA GCTGGCTGAG AACAGGGAGA TCCTGAAGGA GCCTGTGCAT
GGGGTGTA CTGACCCCTC CAAGGACCTG ATTGCTGAGA TCCAGAAGCA GGGCCAGGGC
CAGTGGACCT ACCAAATCTA CCAGGAGCCC TTCAAGAACC TGAAGACTGG CAAGTATGCC
25 AGGATGAGGG GGGCCACAC CAATGATGTG AAGCAGCTGA CTGAGGCTGT GCAGAAGATC
ACCACTGAGT CCATTGTGAT CTGGGGCAAG ACCCCCAAGT TCAAGCTGCC CATCCAGAAG
GAGACCTGGG AGACCTGGTG GACTGAGTAC TGGCAGGCCA CCTGGATCCC TGAGTGGGAG
TTTGTAACA CCCCCCCCCT GGTGAAGCTG TGGTACCAGC TGGAGAAGGA GCCCATTTGTG
GGGGCTGAGA CTTTCTATGT GGCTGGGGCT GCCAACAGGG AGACCAAGCT GGGCAAGGCT
30 GGCTATGTGA CCAACAGGGG CAGGCAGAAG GTGGTGACCC TGACTGACAC CACCAACCAG
AAGACTGCCC TCCAGGCCAT CTACCTGGCC CTCCAGGACT CTGGCCTGGA GGTGAACATT
GTGACTGCCT CCCAGTATGC CCTGGGCATC ATCCAGGCCC AGCCTGATCA GTCTGAGTCT
GAGCTGGTGA ACCAGATCAT TGAGCAGCTG ATCAAGAAGG AGAAGGTGTA CCTGGCCTGG
GTGCCTGCCC ACAAGGGCAT TGGGGGCAAT GAGCAGGTGG ACAAGCTGGT GTCTGCTGGC
35 ATCAGGAAGG TGCTGTTCCCT GGATGGCATT GACAAGGCCC AGGATGAGCA TGAGAAGTAC
CACTCCAACCT GGAGGGCTAT GGCTCTGAC TTCAACCTGC CCCCTGTGGT GGCTAAGGAG
```

ATTGTGGCCT CCTGTGACAA GTGCCAGCTG AAGGGGGAGG CCATGCATGG GCAGGTGGAC  
 TGCTCCCCCTG GCATCTGGCA GCTGGCCTGC ACCCACCTGG AGGGCAAGGT GATCCTGGTG  
 GCTGTGCATG TGGCCTCCGG CTACATTGAG GCTGAGGTGA TCCCTGCTGA GACAGGCCAG  
 GAGACTGCCT ACTTCCTGCT GAAGCTGGCT GGCAGGTGGC CTGTGAAGAC CATCCACACT  
 5 GCCAATGGCT CCAACTTCAC TGGGGCCACA GTGAGGGCTG CCTGCTGGTG GGCTGGCATC  
 AAGCAGGAGT TTGGCATCCC CTACAACCCC CAGTCCCAGG GGGTGGTGGC CTCCATGAAC  
 AAGGAGCTGA AGAAGATCAT TGGGCAGGTG AGGGACCAGG CTGAGCACCT GAAGACAGCT  
 GTGCAGATGG CTGTGTTTCT CCACAACCTT AAGAGGAAGG GGGGCATCGG GGGCTACTCC  
 GCTGGGGAGA GGATTGTGGA CATCATTGCC ACAGACATCC AGACCAAGGA GCTCCAGAAG  
 10 CAGATCACCA AGATCCAGAA CTTCAGGGTG TACTACAGGG ACTCCAGGAA CCCCTGTGG  
 AAGGGCCCTG CCAAGCTGCT GTGGAAGGGG GAGGGGGCTG TGGTGATCCA GGACAACTCT  
 GACATCAAGG TGGTGCCCAG GAGGAAGGCC AAGATCATCA GGGACTATGG CAAGCAGATG  
 GCTGGGGATG ACTGTGTGGC CTCCAGGCAG GATGAGGACT AAAGCCCGG CAGATCT (SEQ ID  
 NO:3).

15 In order to produce the IA-pol-based adenoviral vaccines of the present  
 invention, inactivation of the enzymatic functions was achieved by replacing a total of  
 nine active site residues from the enzyme subunits with alanine side-chains. As  
 shown in Table 1, all residues that comprise the catalytic triad of the polymerase,  
 namely Asp112, Asp187, and Asp188, were substituted with alanine (Ala) residues  
 20 (Larder, et al., *Nature* 1987, 327: 716-717; Larder, et al., 1989, *Proc. Natl. Acad. Sci.*  
 1989, 86: 4803-4807). Three additional mutations were introduced at Asp445,  
 Glu480 and Asp500 to abolish RNase H activity (Asp551 was left unchanged in this  
 IA Pol construct), with each residue being substituted for an Ala residue, respectively  
 (Davies, et al., 1991, *Science* 252:, 88-95; Schatz, et al., 1989, *FEBS Lett.* 257: 311-  
 25 314; Mizrahi, et al., 1990, *Nucl. Acids. Res.* 18: pp. 5359-5353). HIV pol integrase  
 function was abolished through three mutations at Asp626, Asp678 and Glu714.  
 Again, each of these residues has been substituted with an Ala residue (Wiskerchen,  
 et al., 1995, *J. Virol.* 69: 376-386; Leavitt, et al., 1993, *J. Biol. Chem.* 268: 2113-  
 2119). Amino acid residue Pro3 of SEQ ID NO:4 marks the start of the RT gene.  
 30 The complete amino acid sequence of IA-Pol is disclosed herein as SEQ ID NO:4 and  
 Figure 17A-C, as follows:

Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro  
 Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys  
 Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys  
 35 Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala  
 Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg

Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile  
 Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Ala  
 Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys  
 Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile  
 5 Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala  
 Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln  
 Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Ala Ala Leu Tyr Val Gly  
 Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg  
 Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln  
 10 Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys  
 Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val  
 Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile  
 Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr  
 Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu  
 15 Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr  
 Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln  
 Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys  
 Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys  
 Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile  
 20 Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp  
 Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp  
 Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu  
 Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala  
 Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly  
 25 Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Ala  
 Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn  
 Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro  
 Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile  
 Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile  
 30 Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys  
 Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys  
 Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro  
 Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys  
 Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln  
 35 Leu Ala Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His  
 Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly

Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val  
 Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val  
 Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro  
 Tyr Asn Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu  
 5 Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr  
 Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly  
 Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr  
 Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn  
 Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro  
 10 Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn  
 Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp  
 Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp  
 Glu Asp (SEQ ID NO:4) .

As noted above, it will be understood that any combination of the mutations  
 15 disclosed above may be suitable and therefore be utilized as an IA-pol-based  
 adenoviral HIV vaccine of the present invention, either when administered alone or in  
 a combined modality regime and/or a prime-boost regimen. For example, it may be  
 possible to mutate only 2 of the 3 residues within the respective reverse transcriptase,  
 RNase-H, and integrase coding regions while still abolishing these enzymatic  
 20 activities. However, the IA-pol construct described above and disclosed as SEQ ID  
 NO:3, as well as the expressed protein (SEQ ID NO:4;) is preferred. It is also  
 preferred that at least one mutation be present in each of the three catalytic domains.

Another aspect of this portion of the invention are codon optimized HIV-1  
 Pol-based vaccine constructions which comprise a eukaryotic trafficking signal  
 25 peptide such as from tPA (tissue-type plasminogen activator) or by a leader peptide  
 such as is found in highly expressed mammalian proteins such as immunoglobulin  
 leader peptides. Any functional leader peptide may be tested for efficacy. However,  
 a preferred embodiment of the present invention, as with HIV-1 Nef constructs shown  
 herein, is to provide for a HIV-1 Pol mutant adenoviral vaccine construction wherein  
 30 the pol coding region or a portion thereof is operatively linked to a leader peptide,  
 preferably a leader peptide from human tPA. In other words, a codon optimized  
 HIV-1 Pol mutant such as IA-Pol (SEQ ID NO:4) may also comprise a leader peptide  
 at the amino terminal portion of the protein, which may effect cellular trafficking and  
 hence, immunogenicity of the expressed protein within the host cell. As noted in  
 35 Figure 16A-B, a DNA vector which may be utilized to practice the present invention  
 may be modified by known recombinant DNA methodology to contain a leader signal



peptide of interest, such that downstream cloning of the modified HIV-1 protein of interest results in a nucleotide sequence which encodes a modified HIV-1 tPA/Pol protein. In the alternative, as noted above, insertion of a nucleotide sequence which encodes a leader peptide may be inserted into a DNA vector housing the open reading frame for the Pol protein of interest. Regardless of the cloning strategy, the end result is a polynucleotide vaccine which comprises vector components for effective gene expression in conjunction with nucleotide sequences which encode a modified HIV-1 Pol protein of interest, including but not limited to a HIV-1 Pol protein which contains a leader peptide. The amino acid sequence of the human tPA leader utilized herein is as follows: MDAMKRGGLCCVLLLCGAVFVSPSEISS (SEQ ID NO:17). Therefore, another aspect of the present invention is to generate HIV-1 Pol-based vaccine constructions which comprise a eukaryotic trafficking signal peptide such as from tPA. To this end, the present invention relates to a DNA molecule which encodes a codon optimized wt-pol DNA construct wherein the protease (PR) activity is deleted and a human tPA leader sequence is fused to the 5' end of the coding region. A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:5, the open reading frame disclosed herein as SEQ ID NO:6.

To this end, the present invention relates to a DNA molecule which encodes a codon optimized wt-pol DNA construct wherein the protease (PR) activity is deleted and a human tPA leader sequence is fused to the 5' end of the coding region ( herein, "tPA-wt-pol"). A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:5, the open reading frame being contained from an initiating Met residue at nucleotides 8-10 to a termination codon from nucleotides 2633-2635. SEQ ID NO:5 is as follows:

25 GATCACCATG GATGCAATGA AGAGAGGGCT CTGCTGTGTG CTGCTGCTGT GTGGAGCAGT  
CTTCGTTTCG CCCAGCGAGA TCTCCGCCCC CATCTCCCCC ATTGAGACTG TGCCTGTGAA  
GCTGAAGCCT GGCATGGATG GCCCAAGGT GAAGCAGTGG CCCCTGACTG AGGAGAAGAT  
CAAGGCCCTG GTGGAAATCT GCACTGAGAT GGAGAAGGAG GGCAAAATCT CCAAGATTGG  
CCCCGAGAAC CCCTACAACA CCCCTGTGTT TGCCATCAAG AAGAAGGACT CCACCAAGTG  
30 GAGGAAGCTG GTGGACTTCA GGGAGCTGAA CAAGAGGACC CAGGACTTCT GGGAGGTGCA  
GCTGGGCATC CCCCACCCCG CTGGCCTGAA GAAGAAGAAG TCTGTGACTG TGCTGGATGT  
GGGGGATGCC TACTTCTCTG TGCCCTGGA TGAGGACTTC AGGAAGTACA CTGCCTTCAC  
CATCCCCTCC ATCAACAATG AGACCCCTGG CATCAGGTAC CAGTACAATG TGCTGCCCCA  
GGGCTGGAAG GGCTCCCCTG CCATCTTCCA GTCCTCCATG ACCAAGATCC TGGAGCCCTT  
35 CAGGAAGCAG AACCCTGACA TTGTGATCTA CCAGTACATG GATGACCTGT ATGTGGGCTC  
TGACCTGGAG ATTGGGCAGC ACAGGACCAA GATTGAGGAG CTGAGGCAGC ACCTGCTGAG

GTGGGGCCTG ACCACCCCTG ACAAGAAGCA CCAGAAGGAG CCCCCCTTCC TGTGGATGGG  
 CTATGAGCTG CACCCCGACA AGTGGACTGT GCAGCCCATT GTGCTGCCTG AGAAGGACTC  
 CTGGACTGTG AATGACATCC AGAAGCTGGT GGGCAAGCTG AACTGGGCCT CCCAAATCTA  
 CCCTGGCATC AAGGTGAGGC AGCTGTGCAA GCTGCTGAGG GGCACCAAGG CCCTGACTGA  
 5 GGTGATCCCC CTGACTGAGG AGGCTGAGCT GGAGCTGGCT GAGAACAGGG AGATCCTGAA  
 GGAGCCTGTG CATGGGGTGT ACTATGACCC CTCCAAGGAC CTGATTGCTG AGATCCAGAA  
 GCAGGGCCAG GGCCAGTGGA CCTACCAAAT CTACCAGGAG CCCTTCAAGA ACCTGAAGAC  
 TGGCAAGTAT GCCAGGATGA GGGGGGCCCA CACCAATGAT GTGAAGCAGC TGACTGAGGC  
 TGTGCAGAAG ATCACCACCTG AGTCCATTGT GATCTGGGGC AAGACCCCCA AGTTCAAGCT  
 10 GCCCATCCAG AAGGAGACCT GGGAGACCTG GTGGACTGAG TACTGGCAGG CCACCTGGAT  
 CCCTGAGTGG GAGTTTGTGA ACACCCCCC CCTGGTGAAG CTGTGGTACC AGCTGGAGAA  
 GGAGCCCATT GTGGGGGCTG AGACCTTCTA TGTGGATGGG GCTGCCAACA GGGAGACCAA  
 GCTGGGCAAG GCTGGCTATG TGACCAACAG GGCAGGCAG AAGGTGGTGA CCCTGACTGA  
 CACCACCAAC CAGAAGACTG AGCTCCAGGC CATCTACCTG GCCCTCCAGG ACTCTGGCCT  
 15 GGAGGTGAAC ATTGTGACTG ACTCCCAGTA TGCCCTGGGC ATCATCCAGG CCCAGCCTGA  
 TCAGTCTGAG TCTGAGCTGG TGAACCAGAT CATTGAGCAG CTGATCAAGA AGGAGAAGGT  
 GTACCTGGCC TGGGTGCCTG CCCACAAGGG CATTGGGGGC AATGAGCAGG TGGACAAGCT  
 GGTGTCTGCT GGCATCAGGA AGGTGCTGTT CCTGGATGGC ATTGACAAGG CCCAGGATGA  
 GCATGAGAAG TACCACTCCA ACTGGAGGGC TATGGCCTCT GACTTCAACC TGCCCCCTGT  
 20 GGTGGCTAAG GAGATTGTGG CCTCCTGTGA CAAGTGCCAG CTGAAGGGGG AGGCCATGCA  
 TGGGCAGGTG GACTGCTCCC CTGGCATCTG GCAGCTGGAC TGCACCCACC TGGAGGGCAA  
 GGTGATCCTG GTGGCTGTGC ATGTGGCCTC CGGCTACATT GAGGCTGAGG TGATCCCTGC  
 TGAGACAGGC CAGGAGACTG CCTACTTCCT GCTGAAGCTG GCTGGCAGGT GGCCTGTGAA  
 GACCATCCAC ACTGACAATG GCTCCAATT CACTGGGGCC ACAGTGAGGG CTGCCTGCTG  
 25 GTGGGCTGGC ATCAAGCAGG AGTTTGGCAT CCCCTACAAC CCCAGTCCC AGGGGGTGGT  
 GGAGTCCATG AACAAGGAGC TGAAGAAGAT CATTGGGCAG GTGAGGGACC AGGCTGAGCA  
 CCTGAAGACA GCTGTGCAGA TGGCTGTGTT CATCCACAAC TTCAAGAGGA AGGGGGGCAT  
 CGGGGGCTAC TCCGCTGGGG AGAGGATTGT GGACATCATT GCCACAGACA TCCAGACCAA  
 GGAGCTCCAG AAGCAGATCA CCAAGATCCA GAACTTCAGG GTGTACTACA GGGACTCCAG  
 30 GAACCCCTG TGAAGGGCC CTGCCAAGCT GCTGTGGAAG GGGGAGGGGG CTGTGGTGAT  
 CCAGGACAAC TCTGACATCA AGGTGGTGCC CAGGAGGAAG GCCAAGATCA TCAGGGACTA  
 TGGCAAGCAG ATGGCTGGGG ATGACTGTGT GGCCTCCAGG CAGGATGAGG ACTAAAGCCC  
 GGGCAGATCT (SEQ ID NO:5).

The open reading frame of the wild type tPA-pol construct disclosed as SEQ  
 35 ID NO:5 contains 875 amino acids, disclosed herein as SEQ ID NO:6, as follows:  
 Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly

Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ala Pro Ile Ser Pro Ile  
 Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val  
 Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile  
 Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu  
 5 Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr  
 Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln  
 Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys  
 Lys Lys Lys Ser Val Thr Val Leu Asp Val Gly Asp Ala Tyr Phe Ser  
 Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro  
 10 Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu  
 Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr  
 Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr  
 Gln Tyr Met Asp Asp Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln  
 His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly  
 15 Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp  
 Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val  
 Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val  
 Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg  
 Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile  
 20 Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile  
 Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu  
 Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile  
 Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met  
 Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln  
 25 Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe  
 Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr  
 Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro  
 Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala  
 Glu Thr Phe Tyr Val Asp Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly  
 30 Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu  
 Thr Asp Thr Thr Asn Gln Lys Thr Glu Leu Gln Ala Ile Tyr Leu Ala  
 Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Asp Ser Gln Tyr  
 Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu  
 Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu  
 35 Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp  
 Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile

Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala  
 Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val  
 Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln  
 Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Asp Cys Thr His Leu Glu  
 5 Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu  
 Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu  
 Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Asp Asn  
 Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala  
 Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly  
 10 Val Val Glu Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val  
 Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe  
 Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly  
 Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu  
 Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp  
 15 Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly  
 Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro  
 Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly  
 Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:6).

The present invention also relates to a codon optimized HIV-1 Pol mutant  
 20 contained within a recombinant adenoviral vector such as IA-Pol (SEQ ID NO:4)  
 which comprises a leader peptide at the amino terminal portion of the protein, which  
 may effect cellular trafficking and hence, immunogenicity of the expressed protein  
 within the host cell. Any such adenoviral-based HIV-1 DNA pol mutant disclosed in  
 the above paragraphs is suitable for fusion downstream of a leader peptide, such as a  
 25 leader peptide including but not limited to the human tPA leader sequence. Therefore,  
 any such leader peptide-based HIV-1 pol mutant construct may include but is not  
 limited to a mutated DNA molecule which effectively alters the catalytic activity of  
 the RT, RNase and/or IN region of the expressed protein, resulting in at least  
 substantially decreased enzymatic activity one or more of the RT, RNase H and/or IN  
 30 functions of HIV-1 Pol. In a preferred embodiment of this portion of the invention, a  
 leader peptide/HIV-1 DNA pol construct contains a mutation or mutations within the  
 Pol coding region which effectively abolishes RT, RNase H and IN activity. An  
 especially preferable HIV-1 DNA pol construct is a DNA molecule which contains at  
 least one point mutation which alters the active site and catalytic activity within the  
 35 RT, RNase H and IN domains of Pol, such that each activity is at least substantially  
 abolished, and preferably totally abolished. Such a HIV-1 Pol mutant will most likely

comprise at least one point mutation in or around each catalytic domain responsible for RT, RNase H and IN activity, respectfully. An especially preferred embodiment of this portion of the invention relates to a human tPA leader fused to the IA-Pol protein comprising the nine mutations shown in Table 1. The DNA molecule is disclosed  
 5 herein as SEQ ID NO:7 and the expressed tPA-IA Pol protein comprises a fusion junction as shown in Figure 18. The complete amino acid sequence of the expressed protein is set forth in SEQ ID NO:8. To this end, SEQ ID NO:7 discloses the nucleotide sequence which codes for a human tPA leader fused to the IA Pol protein comprising the nine mutations shown in Table 1 (herein, "tPA-opt-IApol"). The open  
 10 reading frame begins with the initiating Met (nucleotides 8-10) and terminates with a "TAA" codon at nucleotides 2633-2635. The nucleotide sequence encoding tPA-IAPol is also disclosed as follows:

GATCACCATG GATGCAATGA AGAGAGGGCT CTGCTGTGTG CTGCTGCTGT GTGGAGCAGT  
 CTTCTGTTTCG CCCAGCGAGA TCTCCGCCCC CATCTCCCCC ATTGAGACTG TGCCTGTGAA  
 15 GCTGAAGCCT GGCATGGATG GCCCCAAGGT GAAGCAGTGG CCCCTGACTG AGGAGAAGAT  
 CAAGGCCCTG GTGGAAATCT GCACTGAGAT GGAGAAGGAG GGCAAAATCT CCAAGATTGG  
 CCCCAGAAC CCCTACAACA CCCCTGTGTT TGCCATCAAG AAGAAGGACT CCACCAAGTG  
 GAGGAAGCTG GTGGACTTCA GGGAGCTGAA CAAGAGGACC CAGGACTTCT GGGAGGTGCA  
 GCTGGGCATC CCCCACCCCG CTGGCCTGAA GAAGAAGAAG TCTGTGACTG TGCTGGCTGT  
 20 GGGGGATGCC TACTTCTCTG TGCCCCTGGA TGAGGACTTC AGGAAGTACA CTGCCTTCAC  
 CATCCCCCTCC ATCAACAATG AGACCCCTGG CATCAGGTAC CAGTACAATG TGCTGCCCCA  
 GGGCTGGAAG GGCTCCCCCTG CCATCTTCCA GTCCCTCCATG ACCAAGATCC TGGAGCCCTT  
 CAGGAAGCAG AACCCTGACA TTGTGATCTA CCAGTACATG GCTGCCCTGT ATGTGGGCTC  
 TGACCTGGAG ATTGGGCAGC ACAGGACCAA GATTGAGGAG CTGAGGCAGC ACCTGCTGAG  
 25 GTGGGGCCTG ACCACCCCTG ACAAGAAGCA CCAGAAGGAG CCCCCCTTCC TGTGGATGGG  
 CTATGAGCTG CACCCCGACA AGTGGACTGT GCAGCCCATT GTGCTGCCTG AGAAGGACTC  
 CTGGACTGTG AATGACATCC AGAAGCTGGT GGGCAAGCTG AACTGGGCCT CCCAAATCTA  
 CCCTGGCATC AAGGTGAGGC AGCTGTGCAA GCTGCTGAGG GGCACCAAGG CCCTGACTGA  
 GGTGATCCCC CTGACTGAGG AGGCTGAGCT GGAGCTGGCT GAGAACAGGG AGATCCTGAA  
 30 GGAGCCTGTG CATGGGGTGT ACTATGACCC CTCCAAGGAC CTGATTGCTG AGATCCAGAA  
 GCAGGGCCAG GGCCAGTGGA CCTACCAAAT CTACCAGGAG CCCTTCAAGA ACCTGAAGAC  
 TGGCAAGTAT GCCAGGATGA GGGGGGCCCA CACCAATGAT GTGAAGCAGC TGA CTGAGGC  
 TGTGCAGAAG ATCACCCTG AGTCCATTGT GATCTGGGGC AAGACCCCCA AGTTCAAGCT  
 GCCCATCCAG AAGGAGACCT GGGAGACCTG GTGGACTGAG TACTGGCAGG CCACCTGGAT  
 35 CCCTGAGTGG GAGTTTGTGA ACACCCCCC CCTGGTGAAG CTGTGGTACC AGCTGGAGAA  
 GGAGCCCATT GTGGGGGCTG AGACCTTCTA TGTGGCTGGG GCTGCCAACA GGGAGACCAA

GCTGGGCAAG GCTGGCTATG TGACCAACAG GGGCAGGCAG AAGGTGGTGA CCCTGACTGA  
 CACCACCAAC CAGAAGACTG CCCTCCAGGC CATCTACCTG GCCCTCCAGG ACTCTGGCCT  
 GGAGGTGAAC ATTGTGACTG CCTCCCAGTA TGCCCTGGGC ATCATCCAGG CCCAGCCTGA  
 TCAGTCTGAG TCTGAGCTGG TGAACCAGAT CATTGAGCAG CTGATCAAGA AGGAGAAGGT  
 5 GTACCTGGCC TGGGTGCCTG CCCACAAGGG CATTGGGGGC AATGAGCAGG TGGACAAGCT  
 GGTGTCTGCT GGCATCAGGA AGGTGCTGTT CCTGGATGGC ATTGACAAGG CCCAGGATGA  
 GCATGAGAAG TACCACTCCA ACTGGAGGGC TATGGCCTCT GACTTCAACC TGCCCCCTGT  
 GGTGGCTAAG GAGATTGTGG CCTCCTGTGA CAAGTGCCAG CTGAAGGGGG AGGCCATGCA  
 TGGGCAGGTG GACTGCTCCC CTGGCATCTG GCAGCTGGCC TGCACCCACC TGGAGGGCAA  
 10 GGTGATCCTG GTGGCTGTGC ATGTGGCCTC CGGCTACATT GAGGCTGAGG TGATCCCTGC  
 TGAGACAGGC CAGGAGACTG CCTACTTCCT GCTGAAGCTG GCTGGCAGGT GGCCTGTGAA  
 GACCATCCAC ACTGCCAATG GCTCCAACCT CACTGGGGCC ACAGTGAGGG CTGCCTGCTG  
 GTGGGCTGGC ATCAAGCAGG AGTTTGGCAT CCCCTACAAC CCCCAGTCCC AGGGGGTGGT  
 GGCTCCATG AACAAGGAGC TGAAGAAGAT CATTGGGCAG GTGAGGGACC AGGCTGAGCA  
 15 CCTGAAGACA GCTGTGCAGA TGGCTGTGTT CATCCACAAC TTCAAGAGGA AGGGGGGAT  
 CGGGGGCTAC TCCGCTGGGG AGAGGATTGT GGACATCATT GCCACAGACA TCCAGACCAA  
 GGAGCTCCAG AAGCAGATCA CCAAGATCCA GAACCTCAGG GTGTACTACA GGGACTCCAG  
 GAACCCCTG TGGAAGGGCC CTGCCAAGCT GCTGTGGAAG GGGGAGGGGG CTGTGGTGTG  
 CCAGGACAAC TCTGACATCA AGGTGGTGCC CAGGAGGAAG GCCAAGATCA TCAGGGACTA  
 20 TGGCAAGCAG ATGGCTGGGG ATGACTGTGT GGCCTCCAGG CAGGATGAGG ACTAAAGCCC  
 GGGCAGATCT (SEQ ID NO:7).

The open reading frame of the tPA-IA-pol construct disclosed as SEQ ID NO:7 contains 875 amino acids, disclosed herein as tPA-IA-Pol and SEQ ID NO:8, as follows:

25 Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly  
 Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ala Pro Ile Ser Pro Ile  
 Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val  
 Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile  
 Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu  
 30 Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr  
 Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln  
 Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys  
 Lys Lys Lys Ser Val Thr Val Leu Ala Val Gly Asp Ala Tyr Phe Ser  
 Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro  
 35 Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu  
 Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr

Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr  
 Gln Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln  
 His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly  
 Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp  
 5 Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val  
 Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val  
 Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg  
 Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile  
 Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile  
 10 Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu  
 Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile  
 Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met  
 Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln  
 Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe  
 15 Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr  
 Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro  
 Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala  
 Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly  
 Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu  
 20 Thr Asp Thr Thr Asn Gln Lys Thr Ala Leu Gln Ala Ile Tyr Leu Ala  
 Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr  
 Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu  
 Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu  
 Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp  
 25 Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile  
 Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala  
 Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val  
 Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln  
 Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu  
 30 Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu  
 Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu  
 Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Ala Asn  
 Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala  
 Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly  
 35 Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val  
 Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe

Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly  
 Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu  
 Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp  
 Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly  
 5 Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro  
 Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly  
 Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:8) .

### EXAMPLE 18

#### 10 CODON OPTIMIZED HIV-1 NEF AND CODON OPTIMIZED HIV-1 NEF MODIFICATIONS

Codon optimized version of HIV-1 Nef and HIV-1 Nef modifications are essentially as described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and PCT International Application PCT/US00/34162, also filed  
 15 December 15, 2000, both documents which are hereby incorporated by reference. As disclosed within the above-mentioned documents, particular embodiments of codon optimized Nef and Nef modifications relate to a DNA molecule encoding HIV-1 Nef from the HIV-1 jf1 isolate wherein the codons are optimized for expression in a mammalian system such as a human. The DNA molecule which encodes this protein  
 20 is disclosed herein as SEQ ID NO:9, while the expressed open reading frame is disclosed herein as SEQ ID NO:10. Another embodiment of Nef-based coding regions for use in the adenoviral vectors of the present invention comprise a codon optimized DNA molecule encoding a protein containing the human plasminogen activator (tpa) leader peptide fused with the NH<sub>2</sub>-terminus of the HIV-1 Nef  
 25 polypeptide. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:11, while the expressed open reading frame is disclosed herein as SEQ ID NO:12. Another modified Nef optimized coding region relates to a DNA molecule encoding optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and  
 30 substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175, herein described as opt nef (G2A, LLAA). The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:13, while the expressed open reading frame is disclosed herein as SEQ ID NO:14. An additional embodiment relates to a DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation  
 35 site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA), comprises an open reading frame which



encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175, herein referred to as opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, while the expressed open reading frame is disclosed herein as SEQ ID NO:16.

5 As disclosed in the above-identified documents (U.S. Application Serial No. 09/738,782 and PCT International Application PCT/US00/34162) and reiterated herein, the following nef-based nucleotide and amino acid sequences which comprise the respective open reading frame are as follows:

1. The nucleotide sequence of the codon optimized version of HIV-1 jfrl nef gene is disclosed herein as SEQ ID NO:9, as shown herein:

10 GATCTGCCAC CATGGGCGGC AAGTGGTCCA AGAGGTCCGT GCCCGGCTGG TCCACCGTGA  
GGGAGAGGAT GAGGAGGGCC GAGCCCGCCG CCGACAGGGT GAGGAGGACC GAGCCCGCCG  
CCGTGGGCGT GGGCGCCGTG TCCAGGGACC TGGAGAAGCA CGGCGCCATC ACCTCCTCCA  
ACACCGCCGC CACCAACGCC GACTGCGCCT GGCTGGAGGC CCAGGAGGAC GAGGAGGTGG  
15 GCTTCCCCGT GAGGCCCCAG GTGCCCCCTGA GGCCCATGAC CTACAAGGGC GCCGTGGACC  
TGTCCTCACTT CCTGAAGGAG AAGGGCGGCC TGGAGGGCCT GATCCACTCC CAGAAGAGGC  
AGGACATCCT GGACCTGTGG GTGTACCACA CCCAGGGCTA CTTCCCCGAC TGGCAGAACT  
ACACCCCCGG CCCCAGCATC AGGTCCCCC TGACCTTCGG CTGGTGCTTC AAGCTGGTGC  
CCGTGGAGCC CGAGAAGGTG GAGGAGGCCA ACGAGGGCGA GAACAACTGC CTGCTGCACC  
20 CCATGTCCCA GCACGGCATC GAGGACCCCG AGAAGGAGGT GCTGGAGTGG AGGTTGCACT  
CCAAGCTGGC CTTCCACCAC GTGGCCAGGG AGCTGCACCC CGAGTACTAC AAGGACTGCT  
AAAGCCCGGG C (SEQ ID NO:9).

Preferred codon usage is as follows: Met (ATG), Gly (GGC), Lys (AAG),  
Trp (TGG), Ser (TCC), Arg (AGG), Val (GTG), Pro (CCC), Thr (ACC), Glu (GAG);  
25 Leu (CTG), His (CAC), Ile (ATC), Asn (AAC), Cys (TGC), Ala (GCC), Gln (CAG),  
Phe (TTC) and Tyr (TAC). For an additional discussion relating to mammalian  
(human) codon optimization, see WO 97/31115 (PCT/US97/02294), which is hereby  
incorporated by reference. See also Figure 19A-B for a comparison of wild type vs.  
codon optimized nucleotides comprising the open reading frame of HIV-Nef.

30 The open reading frame for SEQ ID NO:9 above comprises an initiating  
methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides  
660-662. The open reading frame of SEQ ID NO:9 provides for a 216 amino acid  
HIV-1 Nef protein expressed through utilization of a codon optimized DNA vaccine  
vector. The 216 amino acid HIV-1 Nef (jfrl) protein is disclosed herein as SEQ ID  
35 NO:10, and as follows:

Met Gly Gly Lys Trp Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val

Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg  
 Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu  
 Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp  
 Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val  
 5 Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp  
 Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His  
 Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln  
 Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg  
 Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro  
 10 Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Leu Leu His  
 Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu  
 Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu  
 His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:10).

HIV-1 Nef is a 216 amino acid cytosolic protein which associates with the  
 15 inner surface of the host cell plasma membrane through myristylation of Gly-2  
 (Franchini et al., 1986, *Virology* 155: 593-599). While not all possible Nef functions  
 have been elucidated, it has become clear that correct trafficking of Nef to the inner  
 plasma membrane promotes viral replication by altering the host intracellular  
 environment to facilitate the early phase of the HIV-1 life cycle and by increasing the  
 20 infectivity of progeny viral particles. In one aspect of the invention regarding  
 codon-optimized, protein-modified polypeptides, the nef-encoding region of the  
 adenovirus vector of the present invention is modified to contain a nucleotide  
 sequence which encodes a heterologous leader peptide such that the amino terminal  
 region of the expressed protein will contain the leader peptide. The diversity of  
 25 function that typifies eukaryotic cells depends upon the structural differentiation of  
 their membrane boundaries. To generate and maintain these structures, proteins must  
 be transported from their site of synthesis in the endoplasmic reticulum to  
 predetermined destinations throughout the cell. This requires that the trafficking  
 proteins display sorting signals that are recognized by the molecular machinery  
 30 responsible for route selection located at the access points to the main trafficking  
 pathways. Sorting decisions for most proteins need to be made only once as they  
 traverse their biosynthetic pathways since their final destination, the cellular location  
 at which they perform their function, becomes their permanent residence.  
 Maintenance of intracellular integrity depends in part on the selective sorting and  
 35 accurate transport of proteins to their correct destinations. Defined sequence motifs  
 exist in proteins which can act as 'address labels'. A number of sorting signals have

been found associated with the cytoplasmic domains of membrane proteins. An effective induction of CTL responses often required sustained, high level endogenous expression of an antigen. As membrane-association via myristylation is an essential requirement for most of Nef's function, mutants lacking myristylation, by glycine-to-alanine change, change of the dileucine motif and/or by substitution with a tpa leader sequence as described herein, will be functionally defective, and therefore will have improved safety profile compared to wild-type Nef for use as an HIV-1 vaccine component.

In another embodiment of this portion of the invention, either the DNA vector or the HIV-1 nef nucleotide sequence is modified to include the human tissue-specific plasminogen activator (tPA) leader. As shown in Figure 16A-B, a DNA vector may be modified by known recombinant DNA methodology to contain a leader signal peptide of interest, such that downstream cloning of the modified HIV-1 protein of interest results in a nucleotide sequence which encodes a modified HIV-1 tPA/Nef protein. In the alternative, as noted above, insertion of a nucleotide sequence which encodes a leader peptide may be inserted into a DNA vector housing the open reading frame for the Nef protein of interest. Regardless of the cloning strategy, the end result is a polynucleotide vaccine which comprises vector components for effective gene expression in conjunction with nucleotide sequences which encode a modified HIV-1 Nef protein of interest, including but not limited to a HIV-1 Nef protein which contains a leader peptide. The amino acid sequence of the human tPA leader utilized herein is as follows: MDAMKRGLCCVLLLCGAVFVSPSEISS (SEQ ID NO:17).

It has been shown that myristylation of Gly-2 in conjunction with a dileucine motif in the carboxy region of the protein is essential for Nef-induced down regulation of CD4 (Aiken et al., 1994, *Cell* 76: 853-864) via endocytosis. It has also been shown that Nef expression promotes down regulation of MHCI (Schwartz et al., 1996, *Nature Medicine* 2(3): 338-342) via endocytosis. The present invention relates in part to DNA vaccines which encode modified Nef proteins altered in trafficking and/or functional properties. The modifications introduced into the adenoviral vector HIV vaccines of the present invention include but are not limited to additions, deletions or substitutions to the nef open reading frame which results in the expression of a modified Nef protein which includes an amino terminal leader peptide, modification or deletion of the amino terminal myristylation site, and modification or deletion of the dileucine motif within the Nef protein and which alter function within the infected host cell. Therefore, a central theme of the DNA molecules and recombinant adenoviral HIV vaccines of the present invention is (1)

host administration and intracellular delivery of a codon optimized nef-based  
 adenoviral HIV vaccine; (2) expression of a modified Nef protein which is  
 immunogenic in terms of eliciting both CTL and Th responses; and, (3) inhibiting or  
 at least altering known early viral functions of Nef which have been shown to  
 5 promote HIV-1 replication and load within an infected host. Therefore, the nef  
 coding region may be altered, resulting in a DNA vaccine which expresses a modified  
 Nef protein wherein the amino terminal Gly-2 myristylation residue is either deleted  
 or modified to express alternate amino acid residues. Also, the nef coding region may  
 be altered so as to result in a DNA vaccine which expresses a modified Nef protein  
 10 wherein the dileucine motif is either deleted or modified to express alternate amino  
 acid residues. In addition, the adenoviral vector HIV vaccines of the present  
 invention also relate to an isolated DNA molecule, regardless of codon usage, which  
 expresses a wild type or modified Nef protein as described herein, including but not  
 limited to modified Nef proteins which comprise a deletion or substitution of Gly 2, a  
 15 deletion or substitution of Leu 174 and Leu 175 and/or inclusion of a leader sequence.

Therefore, specific Nef-based constructs further include the following, as  
 exemplification's and not limitations. For example, the present invention relates to an  
 adenoviral vector vaccine which encodes modified forms of HIV-1, an open reading  
 frame which encodes a Nef protein which comprises a tPA leader sequence fused to  
 20 amino acid residue 6-216 of HIV-1 Nef (jfrl) is referred to herein as opt tpanef. The  
 nucleotide sequence comprising the open reading frame of opt tpanef is disclosed  
 herein as SEQ ID NO:11, as shown below:

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CATGGATGCA ATGAAGAGAG GGCTCTGCTG TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT
TTCGCCCAGC GAGATCTCCT CCAAGAGGTC CGTGCCCGGC TGGTCCACCG TGAGGGAGAG
25 GATGAGGAGG GCCGAGCCCG CCGCCGACAG GGTGAGGAGG ACCGAGCCCG CCGCCGTGGG
CGTGGGCGCC GTGTCCAGGG ACCTGGAGAA GCACGGCGCC ATCACCTCCT CCAACACCGC
CGCCACCAAC GCCGACTGCG CCTGGCTGGA GGCCAGGAG GACGAGGAGG TGGGCTTCCC
CGTGAGGCCC CAGGTGCCCC TGAGGCCCAT GACCTACAAG GGCGCCGTGG ACCTGTCCCA
CTTCCTGAAG GAGAAGGGCG GCCTGGAGGG CCTGATCCAC TCCCAGAAGA GGCAGGACAT
30 CCTGGACCTG TGGGTGTACC ACACCCAGGG CTACTTCCCC GACTGGCAGA ACTACACCCC
CGGCCCCGGC ATCAGGTTCC CCCTGACCTT CGGCTGGTGC TTCAAGCTGG TGCCCGTGGA
GCCCCAGAAG GTGGAGGAGG CCAACGAGGG CGAGAACAAC TGCTGCTGC ACCCATGTC
CCAGCACGGC ATCGAGGACC CCGAGAAGGA GTGCTGGAG TGGAGGTTTC ACTCCAAGCT
GGCCTTCCAC CACGTGGCCA GGGAGCTGCA CCCCAGTAC TACAAGGACT GCTAAAGCC
35 (SEQ ID NO:11).
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The open reading frame for SEQ ID NO:11 comprises an initiating methionine

residue at nucleotides 2-4 and a "TAA" stop codon from nucleotides 713-715. The open reading frame of SEQ ID NO:3 provides for a 237 amino acid HIV-1 Nef protein which comprises a tPA leader sequence fused to amino acids 6-216 of HIV-1 Nef, including the dileucine motif at amino acid residues 174 and 175. This 237 amino acid tPA/Nef (jfrl) fusion protein is disclosed herein as SEQ ID NO:12, and is shown as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly  
 Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ser Lys Arg Ser Val Pro  
 Gly Trp Ser Thr Val Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala  
 10 Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val  
 Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala  
 Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu  
 Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr  
 Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu  
 15 Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp  
 Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro  
 Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu  
 Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn  
 Asn Cys Leu Leu His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu  
 20 Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His  
 Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:12).

Therefore, this exemplified Nef protein, Opt tPA-Nef, contains both a tPA leader sequence as well as deleting the myristylation site of Gly-2A DNA molecule encoding HIV-1 Nef from the HIV-1 jfrl isolate wherein the codons are optimized for expression in a mammalian system such as a human.

In another specific embodiment of the present invention, a DNA molecule is disclosed which encodes optimized HIV-1 Nef wherein the open reading frame of a recombinant adenoviral HIV vaccine encodes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175. This open reading frame is herein described as opt nef (G2A,LLAA) and is disclosed as SEQ ID NO:13, which comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662. The nucleotide sequence of this codon optimized version of HIV-1 jfrl nef gene with the above mentioned modifications is disclosed herein as SEQ ID NO:13, as follows:

GATCTGCCAC CATGGCCGGC AAGTGGTCCA AGAGGTCCGT GCCCGGCTGG TCCACCGTGA  
 GGGAGAGGAT GAGGAGGGCC GAGCCCGCCG CCGACAGGGT GAGGAGGACC GAGCCCGCCG  
 CCGTGGGCGT GGGCGCCGTG TCCAGGGACC TGGAGAAGCA CGGCGCCATC ACCTCCTCCA  
 ACACCGCCGC CACCAACGCC GACTGCGCCT GGCTGGAGGC CCAGGAGGAC GAGGAGGTGG  
 5 GCTTCCCCGT GAGGCCCCAG GTGCCCCCTGA GGCCCATGAC CTACAAGGGC GCCGTGGACC  
 TGTCCCACTT CCTGAAGGAG AAGGGCGGCC TGGAGGGCCT GATCCACTCC CAGAAGAGGC  
 AGGACATCCT GGACCTGTGG GTGTACCACA CCCAGGGCTA CTTCCCCGAC TGGCAGAACT  
 ACACCCCCGG CCGCGGCATC AGGTTCCTCC TGACCTTCGG CTGGTGCTTC AAGCTGGTGC  
 CCGTGGAGCC CGAGAAGGTG GAGGAGGCCA ACGAGGGCGA GAACAACCTGC GCCGCCACC  
 10 CCATGTCCCA GCACGGCATC GAGGACCCCG AGAAGGAGGT GCTGGAGTGG AGGTTCGACT  
 CCAAGCTGGC CTTCACCAC GTGGCCAGGG AGCTGCACCC CGAGTACTAC AAGGACTGCT  
 AAAGCCCGGG C (SEQ ID NO:13).

The open reading frame of SEQ ID NO:13 encodes Nef (G2A,LLAA), disclosed herein as SEQ ID NO:14, as follows:

15 Met Ala Gly Lys Trp Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val  
 Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg  
 Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu  
 Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp  
 Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val  
 20 Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp  
 Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His  
 Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln  
 Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg  
 Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro  
 25 Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Ala Ala His  
 Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu  
 Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu  
 His Pro Glu Tyr Tyr Lys Asp Cys Ser (SEQ ID NO:14).

30 An additional embodiment of the present invention relates to another DNA  
 molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation  
 site and dileucine motif have been deleted, as well as comprising a tPA leader peptide.  
 This DNA molecule, opt tpanef (LLAA) comprises an open reading frame which  
 encodes a Nef protein containing a tPA leader sequence fused to amino acid residue  
 6-216 of HIV-1 Nef (jfr1), wherein Leu-174 and Leu-175 are substituted with Ala-174  
 35 and Ala-175 (Ala-195 and Ala-196 in this tPA-based fusion protein). The nucleotide

sequence comprising the open reading frame of opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, as shown below:

CATGGATGCA ATGAAGAGAG GGCTCTGCTG TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT  
 TTCGCCCAGC GAGATCTCCT CCAAGAGGTC CGTGCCCGGC TGGTCCACCG TGAGGGAGAG  
 5 GATGAGGAGG GCCGAGCCCG CCGCCGACAG GGTGAGGAGG ACCGAGCCCG CCGCCGTGGG  
 CGTGGGCGCC GTGTCCAGGG ACCTGGAGAA GCACGGCGCC ATCACCTCCT CCAACACCGC  
 CGCCACCAAC GCCGACTGCG CCTGGCTGGA GGCCAGGAG GACGAGGAGG TGGGCTTCCC  
 CGTGAGGCCC CAGGTGCCCC TGAGGCCCAT GACCTACAAG GCGCCGTGG ACCTGTCCCA  
 CTTCTGAAG GAGAAGGGCG GCCTGGAGGG CCTGATCCAC TCCCAGAAGA GGCAGGACAT  
 10 CCTGGACCTG TGGGTGTACC ACACCCAGGG CTACTTCCCC GACTGGCAGA ACTACACCCC  
 CGGCCCCGGC ATCAGGTTC CCCTGACCTT CGGCTGGTGC TTCAAGCTGG TGCCCGTGGG  
 GCCCCAGAAG GTGGAGGAGG CCAACGAGGG CGAGAACAAC TGCGCCGCC ACCCATGTG  
 CCAGCACGGC ATCAGGACC CCGAGAAGGA GGTGCTGGAG TGGAGGTTG ACTCCAAGCT  
 GGCTTCCAC CACGTGCCA GGGAGCTGCA CCCCAGTAC TACAAGGACT GCTAAAGCCC  
 15 (SEQ ID NO:15).

The open reading frame of SEQ ID NO:7 encoding tPA-Nef (LLAA), disclosed herein as SEQ ID NO:16, is as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly  
 Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ser Lys Arg Ser Val Pro  
 20 Gly Trp Ser Thr Val Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala  
 Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val  
 Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala  
 Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu  
 Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr  
 25 Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu  
 Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp  
 Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro  
 Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu  
 Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn  
 30 Asn Cys Ala Ala His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu  
 Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His  
 Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:16).

An adenoviral vector of the present invention may comprise a DNA sequence,  
 regardless of codon usage, which expresses a wild type or modified Nef protein as  
 35 described herein, including but not limited to modified Nef proteins which comprise a  
 deletion or substitution of Gly 2, a deletion or substitution of Leu 174 and Leu 175

and/or inclusion of a leader sequence. Therefore, partial or fully codon optimized DNA vaccine expression vector constructs are preferred since such constructs should result in increased host expression. However, it is within the scope of the present invention to utilize "non-codon optimized" versions of the constructs disclosed herein,  
 5 especially modified versions of HIV Nef which are shown to promote a substantial cellular immune response subsequent to host administration.

Figure 20A-C show nucleotide sequences at junctions between nef coding sequence and plasmid backbone of nef expression vectors V1Jns/nef (Figure 20A), V1Jns/nef(G2A,LLAA) (Figure 20B), V1Jns/tpanef (Figure 20C) and  
 10 V1Jns/tpanef(LLAA) (Figure 20C, also). 5' and 3' flanking sequences of codon optimized nef or codon optimized nef mutant genes are indicated by bold/italic letters; nef and nef mutant coding sequences are indicated by plain letters. Also indicated (as underlined) are the restriction endonuclease sites involved in construction of respective nef expression vectors. V1Jns/tpanef and V1Jns/tpanef(LLAA) have  
 15 identical sequences at the junctions.

Figure 21 shows a schematic presentation of nef and nef derivatives. Amino acid residues involved in Nef derivatives are presented. Glycine 2 and Leucine 174 and 175 are the sites involved in myristylation and dileucine motif, respectively.

## 20 EXAMPLE 19

### MRKAd5Pol Construction and Virus Rescue

*Construction of vector: shuttle plasmid and pre-adenovirus plasmid* - Key steps performed in the construction of the vectors, including the pre-adenovirus plasmid denoted MRKAd5pol, is depicted in Figure 22. Briefly, the adenoviral shuttle  
 25 vector for the full-length inactivated HIV-1 pol gene is as follows. The vector MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHPA(str.) is a derivative of the shuttle vector used in the construction of the MRKAd5gag adenoviral pre-plasmid. The vector contains an expression cassette with the hCMV promoter (no intronA) and the bovine growth hormone polyadenylation signal. The expression unit has been  
 30 inserted into the shuttle vector such that insertion of the gene of choice at a unique *Bgl*II site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1-/E3+)Cla1 (or MRKpAdHVE3) pre-plasmid. The vector, similar to the original shuttle vector contains the *Pac*I site, extension to the packaging signal region, and extension to the pIX gene. The synthetic  
 35 full-length codon-optimized HIV-1 pol gene was isolated directly from the plasmid pV1Jns-HIV-pol-inact(opt). Digestion of this plasmid with *Bgl* II releases the pol



gene intact (comprising a codon optimized IA pol sequence as disclosed in SEQ ID NO:3). The pol fragment was gel purified and ligated into the MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) shuttle vector at the *Bgl*III site. The clones were checked for the correct orientation of the gene by using  
 5 restriction enzymes *Dra*III/*Not*I. A positive clone was isolated and named MRKpdel+hCMVmin+FL-pol+bGHpA(s). The genetic structure of this plasmid was verified by PCR, restriction enzyme and DNA sequencing. The pre-adenovirus plasmid was constructed as follows. Shuttle plasmid MRKpdel+hCMVmin+FL-pol+bGHpA(S) was digested with restriction enzymes *Pac*I and *Bst*1107 I (or its isoschizomer, *Bst*Z107 I) and then co-transformed into *E. coli* strain BJ5183 with linearized (*Cla*I digested) adenoviral backbone plasmid, MRKpAd(E1-/E3+)*Cla*I.  
 10 The resulting pre-plasmid originally named MRKpAd+hCMVmin+FL-pol+bGHpA(S)E3+ is now referred to as "pMRKAd5pol". The genetic structure of the resulting pMRKAd5pol was verified by PCR, restriction enzyme and DNA  
 15 sequence analysis. The vectors were transformed into competent *E. coli* XL-1 Blue for preparative production. The recovered plasmid was verified by restriction enzyme digestion and DNA sequence analysis, and by expression of the pol transgene in transient transfection cell culture. The complete nucleotide sequence of this pMRKAd5HIV-1pol adenoviral vector is shown in Figure 26 A-AO.

20 *Generation of research-grade recombinant adenovirus* - The pre-adenovirus plasmid, pMRKAd5pol, was rescued as infectious virions in PER.C6<sup>®</sup> adherent monolayer cell culture. To rescue infectious virus, 12 µg of pMRKAd5pol was digested with restriction enzyme *Pac*I (New England Biolabs) and 3.3 µg was transfected per 6 cm dish of PER.C6<sup>®</sup> cells using the calcium phosphate co-  
 25 precipitation technique (Cell Pfect Transfection Kit, Amersham Pharmacia Biotech Inc.). *Pac*I digestion releases the viral genome from plasmid sequences allowing viral replication to occur after entry into PER.C6<sup>®</sup> cells. Infected cells and media were harvested 6 -10 days post-transfection, after complete viral cytopathic effect (CPE) was observed. Infected cells and media were stored at ≤ -60°C. This pol containing  
 30 recombinant adenovirus is referred to herein as "MRKAd5pol". This recombinant adenovirus expresses an inactivated HIV-1 Pol protein as shown in SEQ ID NO:6.

## EXAMPLE 20

### MRKAd5Nef Construction and Virus Rescue

35 *Construction of vector: shuttle plasmid and pre-adenovirus plasmid* - Key steps performed in the construction of the vectors, including the pre-adenovirus

plasmid denoted MRKAd5nef, is depicted in Figure 23. Briefly, as shown in Example 19 above, the vector

MRKpdeIE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) is the shuttle vector used in the construction of the MRKAd5gag adenoviral pre-plasmid. It has been modified to contain the *Pac1* site, extension to the packaging signal region, and extension to the pIX gene. It contains an expression cassette with the hCMV promoter (no intronA) and the bovine growth hormone polyadenylation signal. The expression unit has been inserted into the shuttle vector such that insertion of the gene of choice at a unique *Bgl*11 site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1-/E3+)Cla1 pre-plasmid. The synthetic full-length codon-optimized HIV-1 nef gene was isolated directly from the plasmid pV1Jns/nef (G2A,LLAA). Digestion of this plasmid with *Bgl*11 releases the pol gene intact, which comprises the nucleotide sequence as disclosed in SEQ ID NO:13. The nef fragment was gel purified and ligated into the

MRKpdeIE1+CMVmin+BGHpA(str.) shuttle vector at the *Bgl*11 site. The clones were checked for correction orientation of the gene by using restriction enzyme *Sca*1. A positive clone was isolated and named MRKpdeIE1hCMVminFL-nefBGHpA(s). The genetic structure of this plasmid was verified by PCR, restriction enzyme and DNA sequencing. The pre-adenovirus plasmid was constructed as follows. Shuttle plasmid MRKpdeIE1hCMVminFL-nefBGHpA(s) was digested with restriction enzymes *Pac*1 and *Bst*1107 I (or its isoschizomer, *Bst*Z107 I) and then co-transformed into *E. coli* strain BJ5183 with linearized (*Cla*1 digested) adenoviral backbone plasmid, MRKpAd(E1/E3+)Cla1. The resulting pre-plasmid originally named MRKpdeIE1hCMVminFL-nefBGHpA(s) is now referred to as "pMRKAd5nef". The genetic structure of the resulting pMRKAd5nef was verified by PCR, restriction enzyme and DNA sequence analysis. The vectors were transformed into competent *E. coli* XL-1 Blue for preparative production. The recovered plasmid was verified by restriction enzyme digestion and DNA sequence analysis, and by expression of the nef transgene in transient transfection cell culture. The complete nucleotide sequence of this pMRKAd5HIV-1nef adenoviral vector is shown in Figure 27A-AM.

*Generation of research-grade recombinant adenovirus* - The pre-adenovirus plasmid, pMRKAd5nef, was rescued as infectious virions in PER.C6<sup>®</sup> adherent monolayer cell culture. To rescue infectious virus, 12 µg of pMRKAdnef was digested with restriction enzyme *Pac*1 (New England Biolabs) and 3.3 µg was transfected per 6 cm dish of PER.C6<sup>®</sup> cells using the calcium phosphate co-precipitation technique (Cell Pfect Transfection Kit, Amersham Pharmacia Biotech

Inc.). *PacI* digestion releases the viral genome from plasmid sequences allowing viral replication to occur after entry into PER.C6® cells. Infected cells and media were harvested 6 -10 days post-transfection, after complete viral cytopathic effect (CPE) was observed. Infected cells and media were stored at  $\leq -60^{\circ}\text{C}$ . This nef containing recombinant adenovirus is now referred to as "MRKAd5nef".

#### EXAMPLE 21

##### Construction of Murine CMV Promoter Containing Shuttle Vectors for Inactivated Pol and Nef/G2A,LLAA

10 The murine CMV (mCMV) was amplified from the plasmid pMH4 (supplied by Frank Graham, McMaster University) using the primer set: mCMV (*Not* I) Forward: 5'-ATA AGA ATG CGG CCG CCA TAT ACT GAG TCA TTA GG-3' (SEQ ID NO: 20); mCMV (*Bgl* II) Reverse: 5'-AAG GAA GAT CTA CCG ACG CTG GTC GCG CCT C-3' (SEQ ID NO:21). The underlined nucleotides represent

15 the *Not* I and the *Bgl* II sites respectively for each primer. This PCR amplicon was used for the construction of the mCMV shuttle vector containing the transgene in the E1 parallel orientation. The hCMV promoter was removed from the original shuttle vector (containing the hCMV-gag-bGHpA transgene in the E1 parallel orientation) by digestion with *Not* I and *Bgl* II. The mCMV promoter (*Not* I/*Bgl* II digested PCR

20 product) was inserted into the shuttle vector in a directional manner. The shuttle vector was then digested with *Bgl* II and the gag reporter gene (*Bgl* II fragment) was re-inserted back into the shuttle vector. Several clones were screened for correct orientation of the reporter gene. For the construction of the mCMV-gag in the E1 antiparallel orientation, the mCMV promoter was amplified from the plasmid pMH4

25 using the following primer set: mCMV (*Asc* I) Forward: 5'- ATA AGA ATG GCG CGC CAT ATA CTG AGT CAT TAG G (SEQ ID NO:22); mCMV (*Bgl* II) Reverse: 5' AAG GAA GAT CTA CCG ACG CTG GTC GCG CCT C (SEQ ID NO:23). The underlined nucleotides represent the *Asc* I and *Bgl* II sites, respectively for each primer. The shuttle vector containing the hCMV-gag transgene in the E1 antiparallel

30 orientation was digested with *Asc* I and *Bgl* II to remove the hCMV-gag portion of the transgene. The mCMV promoter (*Asc* I/*Bgl* II digested PCR product) was inserted into the shuttle vector in a directional manner. The vector was then digested with *Bgl* II and the gag reporter gene (*Bgl* II fragment) was re-inserted. Several clones were screened for correct orientation of the reporter gene. For each of the full length

35 IA pol and full length nef/G2A,LLAA genes, cloning was performed using the unique

*Bgl* II site within the mCMV-bGHpA shuttle vector. The pol and nef genes were excised from their respective pV1Jns plasmids by *Bgl* II digestion.

#### EXAMPLE 22

##### 5 Construction of mCMV Full Length Inactivated Pol and Full Length nef/G2A.LLAA Adenovectors

Each of these transgenes of Example 21 were inserted into the modified shuttle vector in both the E1 parallel and E1 anti-parallel orientations. *Pac*I and *Bst*Z110I digestion of each shuttle vector was performed and each specific transgene  
10 fragment containing the flanking Ad5 sequences was isolated and co-transformed with *Cla*I digested MRKpAd5(E3+) or MRKpAd5(E3-) adenovector plasmids via bacterial homologous recombination in BJ5183 *E. coli* cells. Recombinant pre-plasmid adenovectors containing the various transgenes in both the E3- and E3+ versions (and in the E1 parallel and E1 antiparallel orientations) were subsequently  
15 prepared in large scale following transformation into XL-1 Blue *E. coli* cells and analyzed by restriction analysis and sequencing.

#### EXAMPLE 23

##### Construction of hCMV-tpa-nef (LLAA) Adenovector

20 The tpa-nef gene was amplified out from GMP grade pV1Jns-tpanef (LLAA) vector using the primer sets: Tpanef (BamHI) F 5'-ATT GGA TCC ATG GAT GCA ATG AAG AGA GGG (SEQ ID 24); Tpanef (BamHI) R 5'-ATA GGA TCC TTA GCA GTC CTT GTA GTA CTC G (SEQ ID NO:25). The resulting PCR product was digested with *Bam*HI, gel purified and cloned into the *Bgl* II site of MRKAd5CMV-bGHpA shuttle vector (*Bgl* II digested and calf intestinal phosphatase treated).  
25 Clones containing the tpanef (LLAA) gene (see SEQ ID NO:15 for complet coding region) in the correct orientation with respect to the hCMV promoter were selected following *Sca*I digestion. The resulting MRKAd5tpanef shuttle vector was digested with *Pac*I and *Bst*Z110I and cloned into the E3+ MRKAd5 adenovector via bacterial  
30 homologous recombination techniques.

#### EXAMPLE 24

##### Immunogenicity of MRKAd5pol and MRKAd5nef Vaccine

Materials and Methods - Rodent Immunization - Groups of N=10 BALB/c  
35 mice were immunized i.m. with the following vectors: (1) MRKAd5hCMV-IApol (E3+) at either 10<sup>7</sup> vp and 10<sup>9</sup> vp; and (2) MRKAd5hCMV-IApol (E3-) at either

10<sup>7</sup> vp and 10<sup>9</sup> vp. At 7 weeks post dose, 5 of the 10 mice per cohort were boosted with the same vector and dose they initially received. At 3 weeks post the second does, sera and spleens were collected from all the animals for RT ELISA and IFN $\gamma$  ELISpot analyses, respectively. For all rodent immunizations, the Ad5 vectors were  
 5 diluted in 5 mM Tris, 5% sucrose, 75 mM NaCl, 1 mM MgCl<sub>2</sub>, 0.005% polysorbate 80, pH 8.0. The total dose was injected to both quadricep muscles in 50  $\mu$ L aliquots using a 0.3-mL insulin syringe with 28-1/2G needles (Becton-Dickinson, Franklin Lakes, NJ).

Groups of N=10 C57/BL6 mice were immunized i.m. with the following  
 10 vectors: (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+) at either 10<sup>7</sup> vp and 10<sup>9</sup> vp; (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10<sup>7</sup> vp and 10<sup>9</sup> vp; and (3) MRKAd5mCMV-tpanef(LLAA) (E3+) at either 10<sup>7</sup> vp and 10<sup>9</sup> vp. At 7 weeks post dose, 5 of the 10 mice per cohort were boosted with the same vector and dose they initially received. At 3 weeks post the second does, sera and spleens were  
 15 collected from all the animals for RT ELISA and IFN $\gamma$  ELISpot analyses, respectively.

*Non-human Primate immunization* - Cohorts of 3 rhesus macaques (2-3 kg) were vaccinated with the following Ad vectors: (1) MRKAd5hCMV-IApol (E3+) at either 10<sup>9</sup> vp and 10<sup>11</sup> vp dose; and (2) MRKAd5hCMV-IApol (E3-) at either  
 20 10<sup>9</sup> vp and 10<sup>11</sup> vp; (3) MRKAd5hCMV-nef(G2A,LLAA) (E3+) at either 10<sup>9</sup> vp and 10<sup>11</sup> vp; and (4) MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10<sup>9</sup> vp and 10<sup>11</sup> vp. The vaccine was administered to chemically restrained monkeys (10 mg/kg ketamine) by needle injection of two 0.5 mL aliquots of the Ad vectors (in 5 mM Tris, 5% sucrose, 75 mM NaCl, 1 mM MgCl<sub>2</sub>, 0.005% polysorbate 80, pH 8.0)  
 25 into both deltoid muscles. The animals were immunized twice at a 4 week interval (T=0, 4 weeks).

*Murine anti-RT and anti-nef ELISA* - Anti-RT titers were obtained following standard secondary antibody-based ELISA. Maxisorp plates (NUNC, Rochester, NY) were coated by overnight incubation with 100  $\mu$ L of 1  $\mu$ g/mL HIV-1 RT protein  
 30 (Advanced Biotechnologies, Columbia, MD) in PBS. For anti-nef ELISA, 100  $\mu$ L of 1  $\mu$ g/mL HIV-1 nef (Advanced Biotechnologies, Columbia, MD) was used to coat the plates. The plates were washed with PBS/0.05% Tween 20 using Titertek MAP instrument (Huntsville, AL) and incubated for 2 h with 200  $\mu$ L/well of blocking solution (PBS/0.05% tween/1% BSA). An initial serum dilution of 100-fold was  
 35 performed followed by 4-fold serial dilution. 100- $\mu$ L aliquots of serially diluted samples were added per well and incubated for 2 h at room temperature. The plates

were washed and 100  $\mu$ L of 1/1000-diluted HRP-rabbit anti-mouse IgG (ZYMED, San Francisco, CA) were added with 1 h incubation. The plates were washed thoroughly and soaked with 100  $\mu$ L 1,2-phenylenediamine dihydrochloride/hydrogen peroxide (DAKO, Norway) solution for 15 min. The reaction was quenched by adding 100  $\mu$ L of 0.5M H<sub>2</sub>SO<sub>4</sub> per well. OD<sub>492</sub> readings were recorded using Titertek Multiskan MCC/340 with S20 stacker. Endpoint titers were defined as the highest serum dilution that resulted in an absorbance value of greater than or equal to 0.1 OD<sub>492</sub> (2.5 times the background value).

*Non-human primate and murine ELISpot assays* - The enzyme-linked immuno-spot (ELISpot) assay was utilized to enumerate antigen-specific INF $\gamma$ -secreting cells from mouse spleens (Miyahira, et al.1995, *J. Immunol. Methods* 181:45-54) or macaque PBMCs. Mouse spleens were pooled from 5 mice/cohort and single cell suspensions were prepared at  $5 \times 10^6$ /mL in complete RPMI media (RPMI1640, 10% FBS, 2mM L-glutamine, 100U/mL Penicillin, 100 u/mL streptomycin, 10 mM Hepes, 50 uM  $\beta$ -ME). Rhesus PBMCs were prepared from 8-15 mL of heparinized blood following standard Ficoll gradient separation (Coligan, et al, 1998, *Current Protocols in Immunology*. John Wiley & Sons, Inc.). Multiscreen opaque plates (Millipore, France) were coated with 100  $\mu$ L/well of either 5  $\mu$ g/mL purified rat anti-mouse IFN- $\gamma$  IgG1, clone R4-6A2 (Pharmingen, San Diego, CA), or 15  $\mu$ g/mL mouse anti-human IFN- $\gamma$  IgG<sub>2a</sub> (Cat. No. 1598-00, R&D Systems, Minneapolis, MN) in PBS at 4°C overnight for murine or monkey assays, respectively. The plates were washed with PBS/penicillin/streptomycin and blocked with 200  $\mu$ L/well of complete RPMI media for 37 °C for at least 2 h.

To each well, 50  $\mu$ L of cell samples ( $4-5 \times 10^5$  cells per well) and 50  $\mu$ L of the antigen solution were added. To the control well, 50  $\mu$ L of the media containing DMSO were added; for specific responses, either selected peptides or peptide pools (4  $\mu$ g/mL per peptide final concentration) were added. For BALB/c mice immunized with the pol constructs, stimulation was conducted using a pool of CD4<sup>+</sup>-epitope containing 20-mer peptides (aa21-40, aa411-430, aa641-660, aa731-750, aa771-790) or a pool of CD8<sup>+</sup>-epitope containing peptides (aa201-220, aa311-330, aa781-800). For C57/BL6 mice immunized with the nef construct, either aa51-70 (CD8<sup>+</sup> T cell epitope) or aa81-100 (CD4<sup>+</sup>) peptide derived from the nef sequence was added for specific stimulation. In monkeys, the responses against pol were evaluated using two pools (L and R) of 20-aa peptides that encompass the entire pol sequence and overlap by 10 amino acids. In monkeys vaccinated with the nef constructs, a single pool containing 20-mer peptides covering the entire HIV-1 nef sequence and overlapping

by 10 aa was used. Each sample/antigen mixture was performed in triplicate wells for murine samples or in duplicate wells for rhesus PBMCs. Plates were incubated at 37°C, 5% CO<sub>2</sub>, 90% humidity for 20-24 h. The plates were washed with PBS/0.05% Tween 20 and incubated with 100 µL/well of either 1.25 µg/mL biotin-conjugated rat  
 5 anti-mouse IFN-γ mAb, clone XMG1.2 (Pharmingen) or of 0.1 µg/mL biotinylated anti-human IFN-gamma goat polyclonal antibody (R&D Systems) at 4°C overnight. The plates were washed and incubated with 100 µL/well 1/2500 dilution of streptavidin-alkaline phosphatase conjugate (Pharmingen) in PBS/0.005% Tween/5% FBS for 30 min at 37 °C. Spots were developed by incubating with 100 µL/well 1-  
 10 step NBT/BCIP (Pierce Chemicals) for 6-10 min. The plates were washed with water and allowed to air dry. The number of spots in each well was determined using a dissecting microscope and the data normalized to 10<sup>6</sup> cell input.

*Non-human Primate anti-RT ELISA* - The pol-specific antibodies in the monkeys were measured in a competitive RT EIA assay, wherein sample activity is  
 15 determined by the ability to block RT antigen from binding to coating antibody on the plate well. Briefly, Maxisorp plates were coated with saturating amounts of pol positive human serum (#97111234). 250 µL of each sample is incubated with 15 µL of 266 ng/mL RT recombinant protein (in RCM 563, 1% BSA, 0.1% tween, 0.1% NaN<sub>3</sub>) and 20 µL of lysis buffer (Coulter p24 antigen assay kit) for 15 min at room  
 20 temperature. Similar mixtures are prepared using serially diluted samples of a standard and a negative control which defines maximum RT binding. 200 µL/well of each sample and standard were added to the washed plate and the plate incubated 16-24 h at room temperature. Bound RT is quantified following the procedures described in Coulter p24 assay kit and reported in milliMerck units per mL arbitrarily defined  
 25 by the chosen standard.

*Results - Rodent Studies* - BALB/c mice (n=5 mice/cohort) were immunized once or twice with varying doses of MRKAd5hCMV-IApol(E3+) and MRKAd5hCMV-IApol(E3-). At 3 weeks after the second dose, Anti-pol IgG levels were determined by an ELISA assay using RT as a surrogate antigen. Cellular  
 30 response were quantified via IFNγ ELISpot assay against pools of pol-epitope containing peptides. The results of these assays are summarized in Table 10. The results indicate that the mouse vaccinees exhibited detectable anti-RT IgGs with an adenovector dose as low as 10<sup>7</sup> vp. The humoral responses are highly dose-dependent and are boostable with a second immunization. One or two doses of either  
 35 pol vectors elicit high frequencies of antigen-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells; the responses are weakly dose-dependent but are boostable with a second immunization.

Table 10. Immunogenicity of MRKAd5pol Vectors in BALB/c mice.

Group	Vaccine	Dose	No. of Doses	Anti-RT IgG Titers <sup>a</sup>			SFC/10 <sup>6</sup> cells <sup>b</sup>		
				GMT	+SE	-SE	Medium	CD4+ peptide pool	CD8+ peptide pool
1	MRKAd5hCMVFLpol (E3+)	10 <sup>7</sup> vp	2 1	310419 919	301785 372	153020 265	1(1) 1(1)	75(4) 72(9)	2313(67) 533(41)
2	MRKAd5hCMVFLpol (E3+)	10 <sup>9</sup> vp	2 1	1838400 <sup>b</sup> 713155	0 528520	0 303555	2(2) 1(1)	114(9) 48(7)	2083(182) 733(69)
3	MRKAd5hCMVFLpol (E3-)	10 <sup>7</sup> vp	2 1	310419 8400	386218 14013	172097 4393	0(0) 10(8)	223(7) 141(21)	2807(27) 409(28)
4	MRKAd5hCMVFLpol (E3-)	10 <sup>9</sup> vp	2 1	1838400 <sup>b</sup> 1241675 <sup>b</sup>	0 396725	0 300861	1(1) 0(0)	180(13) 39(13)	2385(11) 833(83)
5	Naïve	none	none	57	9	7	9(2)	11(4)	10(1)

<sup>a</sup>GMT, geometric mean titer of the cohort of 5 mice; SE, standard error of the geometric mean<sup>b</sup>Near or at the upper limit of the serial dilution; hence, could be greater than this value<sup>c</sup>No. of Spot-forming Cells per million splenocytes; mean values of triplicates are reported along with standard errors in parenthesis.

- 5 C57/BL6 mice were immunized once or twice with varying doses of MRKAd5hCMV-nef(G2A,LLAA) (E3+), MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10<sup>7</sup> vp and (3) MRKAd5mCMV-tpanef(LLAA) (E3+) at either 10<sup>7</sup> vp and 10<sup>9</sup> vp. The immune response were analyzed using similar protocols and the results are listed in Table 11. While anti-nef IgG responses could not be detected in this
- 10 model system with any of the constructs, there are strong indications of a cellular immunity generated against nef using the ELISpot assay.

Table 11. Immunogenicity of MRKAd5nef Vectors in C57/BL6 mice.

Group	Vaccine	Dose	No. of Doses	Anti-nef IgG Titers <sup>a</sup>			SFC/10 <sup>6</sup> cells <sup>b</sup>		
				GMT	+SE	-SE	Medium	aa51-70 CD8+	aa81-100 CD4+
1	MRKAd5hCMVFLnef (E3+)	10 <sup>7</sup> vp	2 1	174 132	70 42	50 32	1(1) 0(0)	23(1) 0(0)	1(1) 0(0)
2	MRKAd5hCMVFLnef (E3+)	10 <sup>9</sup> vp	2 1	174 132	70 42	50 32	0(0) 1(1)	61(7) 62(7)	4(2) 3(1)
3	MRKAd5mCMVFLnef (E3+)	10 <sup>7</sup> vp	2 1	132 115	42 46	32 33	3(1) 3(2)	15(5) 3(2)	5(2) 4(2)
4	MRKAd5mCMVFLnef (E3+)	10 <sup>9</sup> vp	2 1	132 132	42 42	32 32	4(2) 2(1)	83(13) 29(2)	5(1) 4(0)
5	MRKAd5mCMVtpanef(E3+)	10 <sup>7</sup> vp	2 1	132 100	42 0	32 0	3(2) 3(1)	14(2) 13(4)	5(1) 10(3)
6	MRKAd5mCMVtpanef(E3+)	10 <sup>9</sup> vp	2 1	230 115	170 46	88 33	3(2) 7(1)	145(29) 151(14)	4(0) 10(0)
7	Naïve	none	none	152	78	52	21(2)	18(6)	26(3)

<sup>a</sup>GMT, geometric mean titer of the cohort of 5 mice; SE, standard error of the geometric mean<sup>b</sup>No. of spot-forming cells per million splenocytes; mean values of triplicates are reported along with standard errors in parenthesis.

15

*Monkey Studies* - Cohorts of 3 rhesus macaques were immunized with 2 doses of MRKAd5hCMV-IAPol(E3+) and MRKAd5hCMV-IAPol(E3-). The number of antigen-specific T cells (per million PBMCs) were enumerated using one of two



- peptide pools (L and R) that cover the entire pol sequence; the results are listed in Table 12. Moderate-to-strong T cell responses were detected in the vaccinees using either constructs even at a low dose of  $10^9$  vp. Longitudinal analyses of the anti-RT antibody titers in the animals suggest that the pol transgene product is expressed efficiently to elicit a humoral response (Table 13). It would appear that generally higher immune responses were observed in animals that received the E3- construct compared to the E3+ virus.

Table 12. Pol-specific T Cell Responses in MRKAd5pol Immunized Rhesus Macaques.

Vaccine (T=0,4 wks)	Monkey #	Prebleed			T=4			T=7			T=16		
		Mock	Pol L	Pol R	Mock	Pol L	Pol R	Mock	Pol L	Pol R	Mock	Pol L	Pol R
MRKAd5hCMV-IAPol(E3+) $10^{11}$ vp	99C100	1	0	0	1	38	31	0	52	148	0	49	715
	99C215	1	2	2	10	98	249	1	109	305	22	88	250
	99D201	5	5	4	6	149	85	0	40	35	0	35	18
MRKAd5hCMV-IAPol(E3+) $10^9$ vp	99D212	0	2	0	4	331	114	0	58	14	0	6	8
	99D180	0	4	2	0	19	182	4	39	156	5	38	108
	99C201	8	5	21	6	62	62	0	18	32	1	14	65
MRKAd5hCMV-IAPol(E3-) $10^{11}$ vp	99D239	5	2	2	20	82	172	1	68	114	9	21	40
	99C186	4	12	8	5	120	421	2	271	489	16	875	530
	99C084	1	8	9	8	84	484	0	14	238	1	24	284
MRKAd5hCMV-IAPol(E3-) $10^9$ vp	CC7C	10	10	8	12	724	745	4	322	376	4	188	178
	CD1G	2	0	1	5	474	468	0	232	212	0	101	121
	CD11	8	6	12	10	98	110	5	60	80	8	25	34
Naïve	089Q	nd	nd	nd	nd	nd	nd	4	2	2	2	1	2

nd, not determined

Reported are SFC per million PBMCs; mean of duplicate wells.

Table 13. Anti-RT Ig Levels in MRKAd5pol Immunized macaques.

RT ANTIBODY ASSAY TITERS IN mMU/mL				
Vaccine/Monkey Tag	T=4	T=7	T=12	T=16
MRKAd5hCMV-IAPol(E3+), $10^{11}$ vp				
99C100	61	1999	5928	4768
99C215	81	1541	2356	2767
99D201	53	336	539	387
MRKAd5hCMV-IAPol(E3+), $10^9$ vp				
99D212	10	40	49	68
99D180	<10	36	79	93
99C201	<10	37	71	76
MRKAd5hCMV-IAPol(E3-), $10^{11}$ vp				
99D239	44	460	1234	1015
99C186	21	233	480	345
99C084	235	2637	2858	1626
MRKAd5hCMV-IAPol(E3-), $10^9$ vp				
CC7C	32	175	306	235
CD1G	20	140	273	419
CD11	15	112	149	237

When rhesus macaques were immunized i.m. with two doses of MRKAd5nef

- 5 constructs, vigorous T cell responses ranging from 100 to as high as 1100 per million were observed in 8 of 12 vaccinees (Table 14). The efficacies of the mCMV- and hCMV- driven nef constructs are comparable on the basis of the data generated thus far.

10 Table 14. Nef-specific T cell Responses in MRKAd5nef Immunized Rhesus Macaques.

Vaccine (T=0,4 wks)	Monk #	Pre		T=4		T=7		T=16	
		Mock	Nef	Mock	Nef	Mock	Nef	Mock	Nef
MRKAd5hCMV-nef(G2A,LLAA) (E3+) 10 <sup>6</sup> 11 vp	CD2D	0	4	31	440	4	368	1	251
	CC7B	0	0	2	521	0	178	1	1522
	CC61	2	9	31	112	0	108	11	100
MRKAd5hCMV-nef(G2A,LLAA) (E3+) 10 <sup>6</sup> 9 vp	CC2K	9	9	6	62	0	35	0	15
	CD15	5	4	30	998	2	586	0	434
	CD16	6	1	6	1146	0	369	1	212
MRKAd5mCMV-nef(G2A,LLAA) (E3+) 10 <sup>6</sup> 11 vp	99D191	1	5	4	614	0	298	2	419
	99D144	4	6	5	434	0	1100	2	932
	99C193	1	2	1	58	1	22	0	64
MRKAd5mCMV-nef(G2A,LLAA) (E3+) 10 <sup>6</sup> 9 vp	99D224	1	11	14	231	1	125	0	70
	99D250	8	9	4	108	0	54	0	5
	99C120	1	6	20	299	0	92	0	79
Naïve	083Q	nd	nd	18	22	4	5	2	1

#### EXAMPLE 25

- 15 Comparison of Clade B vs. Clade C T Cell Responses in HIV-Infected Subjects

PBMC samples collected from two dozens of patients infected with HIV-1 in US were tested in ELISPOT assays with peptide pools of 20-mer peptides overlapping by 10 amino acids. Four different peptide pools were tested for cross-clade recognition, and they were either derived from a clade B-based isolate (gag H-b; nef-  
 20 b) or a clade C-based isolate (gag H-c, nef-c). Data in Table 15 shows that T cells from these patients presumably infected with clade B HIV-1 could recognize clade C gag and nef antigens in ELISPOT assay. Correlation analysis further demonstrated that these T cell responses against clade C gag peptide pool were about 60% of the clade B counterpart (Figure 24), while the T cell responses against clade C nef were  
 25 about 85% of the clade B counterpart (Figure 25). These results suggest that cellular immune responses generated in patients infected with clade B HIV-1 can recognize gag and nef antigens derived from clade C HIV-1. These data show that a HIV vaccine, such as a DNA or MRKAd5-based adenoviral vaccine expressing a clade B

gag and/or nef antigen will potentially have the ability to provide a prophylactic and/or therapeutic advantage on a global scale.

5

Table 15  
Responses Shown as the Number of gIFN-Secreting T Cells per Million PBMCs

subject	bleed date	gag epitope # (from mapping)	mock	gag H-b	gagH-c	nef-b	nef-c
#100	19-Jul-99	12	10	3950	1385	1295	1300
#101	25-Jul-99	3	15	3885	1280	na	1020
#102	25-Jul-99	4	15	1740	850	1255	1785
#104	7-Jun-99	2	5	1355	1185	na	1060
#107	11-Oct-99	2	25	3305	2795	670	870
#405	11-Jul-99	2	15	4575	3180	1700	1500
#501	19-Jul-99	2	15	1100	570	3365	3460
#505	18-Jul-99	5	10	2145	1725	1235	na
#506	28-Feb-99	2	25	150	45	400	610
#701	28-Mar-99	5	30	7620	4775	3320	2780
#709	17-May-99	3	15	2785	1945	1090	1630
#710	24-May-99	4	5	1055	1080	2210	2140

10

#### EXAMPLE 26

#### Characterization and Production of MRKAd5pol and MRKAd5nef Vectors in Roller Bottles

*Expansion of nef and pol Adenovectors* - Nef and pol CsCl purified MRKAd5 seeds were used to infect roller bottles to produce P4 virus to be used as a seed for further experiments. P4 MRKAd5 pol and nef vectors were used to infect roller bottles at an MOI 280 vp/cell, except for hCMV-tpa-nef [E3+] which was infected at an MOI of 125 due to low titers of seed obtained at P4.

20

Table 16 Viral particle concentrations for P5 nef and pol adenovectors

Adenovector	AEX Titer (10 <sup>10</sup> vp/ml culture)	AEX Titer (10 <sup>4</sup> vp/cell)	Amplification Ratio
hCMV-FL-nef [E3+]	1.1	0.9	30
mCMV-FL-nef [E3+]	2.2	2.1	75
hCMV-tpa-nef [E3+]	0.07	0.1	5
mCMV-tpa-nef [E3+]	1.3	0.9	35
hCMV-FL-pol [E3+]	2.7	2.1	75
hCMV-FL-pol [E3-]	1.9	1.3	45

- 5 *Roller Bottle Passaging* - Passaging of the *pol* and *nef* constructs continued through passage seven. Cell-associated (freeze/thaw lysis) and whole broth (triton-lysis) titers obtained in all passages were very consistent. In general, MRKAd5pol is ca. 70% as productive as MRKAd5gag while MRKAd5nef is ca. 25% as productive as MRKAd5gag. Samples of P7 virus for both constructs were analyzed by V&CB by
- 10 restriction digest analysis and did not show any rearrangements.

Table 17. Passage Six Viral Productivity for MRKAd5pol and MRKAd5nef

		Xviable ( $10^6$ cells/ml), Viability (%)		Cell Passage Number	AEX Titer (Cell Associated) $10^{10}$ vp/ml culture	Titer $10^4$ vp/cell	Amplification Ratio	Triton Lysis Titer $10^{10}$ vp/ml culture
		Infection	Harvest					
hCMV-FL-nef [E3+]	pool	1.22, 85%		62	0.8	0.7	25	1.6
	1		0.99, 62%					
	2		1.10, 72%					
hCMV-FL-pol [E3+]	pool	1.42, 89%		62	4.5	3.2	115	7.0
	1		1.22, 70%					
	2		1.42, 74%					

15 Table 18. Passage Seven Viral Productivity for MRKAd5pol and MRKAd5nef

		Xviable ( $10^6$ cells/ml), Viability (%)		Cell Passage Number	AEX Titer (Cell Associated) $10^{10}$ vp/ml culture	Titer $10^4$ vp/cell	Amplification Ratio	Triton Lysis Titer $10^{10}$ vp/ml culture
		Infection	Harvest					
hCMV-FL-nef [E3+]	Pool	1.33, 90%		66	1.0	0.8	29	2.1
	1		0.96, 70%					
	2		1.18, 73%					
hCMV-FL-pol [E3+]	Pool	0.90*, 90%		56	4.2	4.7	168	6.5
	1		1.18, 88%					
	2		1.04, 80%					

- MRKAd5nef and MRKAd5pol Viral Production Kinetics* - A timecourse experiment was carried out in roller bottles to determine if the viral production kinetics of the MRKAd5pol and MRKAd5nef vectors were similar to those of MRKAd5gag. PER.C6<sup>®</sup> cells in roller bottle cultures were infected at an MOI of 280 vp/cells with P5 MRKAd5pol, P5 MRKAd5nef and P7 MRKAd5gag; for each adenovector, two infected bottles were sampled at 24, 36, 48, and 60 hours post infection. In addition, two bottles were left unsampled until 48 hpi when they were harvested under the Phase I process conditions. The anion-exchange HPLC viral
- 20 particle concentrations of the freeze-thaw recovered cell associated virus at the 24, 36,
- 25

48, and 60 hpi timepoints are shown in Figure 29A-B. The QPA titers show a similar trend (data not shown).

*Comparison of hCMV- and mCMV-FL-nef* - As the titers obtained with the MRKAd5nef construct (hCMV-FL-nef) were lower than those obtained with MRKAd5gag or MRKAd5pol, a viral productivity comparison experiment was performed with mCMV-FL-nef. For each of the two adenovectors (hCMV- and mCMV-FL-nef), two roller bottles were infected at an MOI of 280 vp/cell with passage five clarified lysate. The macroscopic and microscopic observations of the four roller bottles were identical at the time of harvest. Analysis of the clarified lysate produced indicated a higher viral particle concentration in the bottles infected with mCMV-FL-nef, as shown in Table 19. It is stipulated that the higher productivity with mCMV promoter driven nef vector is due to lower nef expression levels in PER.C6® cells- experiments are underway at V&CB to measure nef expression levels.

Table 19. Passage Six Viral Productivity Comparison of hCMV- and mCMV-FL-nef

		Xv (10 <sup>6</sup> cells/ml), Viability (%)		Cell Passage	AEX Titer	Titer	Amplification	Trion Lysis Titer
		Infection	Harvest	Number	10 <sup>10</sup> vp/ml culture	10 <sup>6</sup> vp/cell	Ratio	10 <sup>10</sup> vp/ml culture
hCMV-FL-nef (MRKAd5nef)	Pool	1.11, 91%		60	1.5	1.4	50	2.8
	1		1.23, 75%					
	2		1.34, 74%					
mCMV-FL-nef	Pool	1.11, 91%		60	2.3	2.1	75	4.6
	1		1.49, 84%					
	2		1.18, 77%					

## EXAMPLE 27

### Characterization and Large Scale Production of MRKAd5nef Virus in Bioreactors

*Materials and Methods* - The experiment of the present example was run twice under the following conditions: 36.5°C, DO 30%, pH 7.30, 150rpm agitation rate, no sparging, Life Technologies (Gibco, Invitrogen) 293 SFM II (with 6mM L-glutamine), 0.5M NaOH as base for pH control. During the first run (B20010115), two 10L stirred vessel bioreactors were inoculated with PER.C6® cells at a concentration of 0.2x10<sup>6</sup> cells/ml. Cells were grown until they reached a cell concentration of approximately 1x10<sup>6</sup> cells/ml. The cells were infected with uncloned MRKAd5nef (G2A,LLAA) at a MOI of 280 virus particles (vp)/cell. For the second batch (B20010202), the same procedure as the first run was used, except the cells

- were infected with cloned MRAd5nef. During both runs, the bioreactors were harvested 48 hours post-infection. Samples were taken and virus concentrations were determined from whole broth (with triton lysis), supernatant, and cell pellets (3 X freeze/thaw) with the AEX and QPA assays. Metabolites were measured with BioProfile 250 throughout the process.

Table 20: Experimental Conditions

Temperature	36.5 °C
DO	30%
PH	7.30
Agitation	150 rpm
Sparging	None

Table 21: Virus source used for experiments.

10

Run	Batch ID	Cloned/Uncloned MRKAd5nef	MOI (vp/cells)
#1	B20010115-1	Uncloned	280
	B20010115-2	Uncloned	280
#2	B20010202-1	Cloned	280
	B20010202-2	Cloned	280

*Results* - Table 22 and 23 show an the ability to scale up production of MRKAd5nef by growth in a bioreactor.

15

Table 22: Virus Concentration as measured by the AEX assay

Run	Batch ID	Cloned/Uncloned MRKAd5nef	Virus Concentration @ 48hpi (1x10 <sup>13</sup> vp/L)			
			Supernatant	Clarified Lysate	Total	Triton Lysate
#1	B20010115-1	Uncloned	0.72	3.26	3.98	5.76
	B20010115-2	Uncloned	0.38	1.67	2.05	2.46
#2	B20010202-1	Cloned	0.80	6.00	6.80	8.88
	B20010202-2	Cloned	0.50	6.00	6.50	8.47

Table 23: Virus Titers as measured by the QPA assay

Run	Batch ID	Cloned/Uncloned MRKAd5nef	Virus Concentration @ 48hpi (1x10 <sup>11</sup> IU/L)				
			Whole Broth	Supernatant	Clarified Lysate	Total	Triton Lysate
#1	B20010115-1	Uncloned	0.13	1.12	1.76	2.88	11.28
	B20010115-2	Uncloned	0.14	0.73	1.54	2.27	5.86
#2	B20010202-1	Cloned	0.14	0.97	1.62	2.69	11.89
	B20010202-2	Cloned	0.14	1.17	1.70	2.97	12.47

20

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art

from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

#### EXAMPLE 28

##### 5 MRKAd5HIV-1gag Boosting of DNA-Primed Animals

Groups of 3-5 rhesus macaques were immunized with (a) 5 mgs of V1Jns-Flgag (pV1JnsCMV(no intron)-FL-gag-bGHpA), (b) 5 mgs of V1Jns-Flgag formulated with 45 mgs of a non-ionic block copolymer CRL1005, or (c) 5 mgs of  
10 V1Jns-Flgag formulated with 7.5 mgs of CRL1005 and 0.6 mM benzalkonium chloride at weeks 0, 4, and 8. All animals received a single dose of  $10^7$  viral particles (vp) of the MRKAd5HIV-1gag at week 26. Note:  $10^7$  is too low to prime or boost effectively when used as a single modality (dose is selected to mimic preexposure to adenovirus); see Figure 32.

15 Blood samples were collected from all animals at several time points and peripheral blood mononuclear cells (PBMCs) were prepared using standard Ficoll method. The PBMCs were counted and analyzed for gamma-interferon secretion using the ELISpot assay (Table 24). For each monkey, the PBMCs were incubated overnight either in the absence (medium) or presence of a pool (called "gag H") of 50  
20 20-aa long peptides that encompass the entire HIV-1 gag sequence.

The results indicate that MRKAd5HIV-1gag was very effective in boosting the T cell immune responses in these monkeys. At week 28 or 2 weeks after the viral boost, the number of gag-specific T cells per million PBMCs increased 2-48 fold compared to the levels observed at week 24 or 2 weeks prior to the boost.

25 The PBMCs were also analyzed by intracellular gamma-interferon staining prior to (at week 10) and after the MRKAd5gag boost (at week 30). The results for select animals are shown on Figure 31. The results indicate that (a) immunization with DNA/adjuvant formulation elicited T cell responses which can either be balanced,  $CD4^+$ -biased or  $CD8^+$ -biased, and (b) boosting with the MRKAd5gag  
30 construct produced in all cases a strongly  $CD8^+$ -biased response. These results suggest that boosting with MRKAd5HIV-1gag construct is able to improve the levels of antigen-specific  $CD8^+$  T cells.

Table 24. Boosting of DNA/Adjuvant-Primed Rhesus Monkeys with MRKAd5gag  
Number of SFC/million PBMCs

Group	Priming T=0, 4, 8 wks DNA/5 mgs PBS (D101)	Boost T=26 wks MRKAd5gag(E3+) 10 <sup>-7</sup> vp	T=0		T=4		T=6		T=10		T=17		T=24		T=28		T=30	
			Medium	gag H	Medium	gag H	Medium	gag H	Medium	gag H	Medium	gag H	Medium	gag H	Medium	gag H	Medium	gag H
1			NA	NA	3	35	15	71	4	224	8	115	6	65	19	958	0	316
			0	0	0	15	0	46	0	68	0	75	0	35	3	1705	1	755
			5	11	0	36	3	51	3	46	2	89	8	65	10	989	0	395
2			0	4	1	60	0	111	5	270	4	260	8	232	3	959	19	1345
			4	0	1	101	0	254	0	791	5	452	0	321	0	1815	1	1099
			9	8	1	10	4	71	4	154	8	104	5	65	11	838	6	241
			NA	NA	0	31	0	288	0	530	19	374	9	251	8	1549	20	1734
			9	12	4	36	1	119	0	439	0	425	0	316	4	1229	5	1354
3			10	4	1	59	5	264	19	425	6	105	9	205	18	565	8	404
			1	0	3	121	1	135	1	270	5	130	1	105	14	1384	10	978
			8	6	0	6	3	119	0	274	6	282	1	208	0	636	1	828
			4	3	0	26	1	91	0	139	0	184	1	62	5	643	1	349
			1	0	0	136	0	316	1	609	6	626	1	759	0	2278	4	1831
4			3	0	0	0	1	0	0	0	0	1	1	2	3	0	0	0
			None	None	None	None	None	None	None	None	None	None	None	None	None	None	None	None

NA, not available



## EXAMPLE 29

## Construction of gagpol fusion for MRKAd5gagpol fusion constructs

The open reading frames for the codon-optimized HIV-1 gag gene was fused  
5 directly to the open reading frame of the IA pol gene (consisting of RT, RNaseH and  
integrase domains) by stepwise PCR. Because the gene (SEQ ID NO: 38) does not  
include the protease gene and the frameshift sequence, it encodes a single polypeptide  
of the combined size of p55, RT, RNase H and integrase (1350 amino acids; SEQ ID  
NO: 39).

10 The fragment that extends from the BstEII site within the gag gene to the last  
non-stop codon was ligated via PCR to a fragment that extends from the start codon  
of the IAPol to a unique BamHI site. This fragment was digested with BstEII and  
BamHI. Construction of gag-IAPol fusion was achieved via three-fragment ligation  
involving the PstI-BstEII gag digestion fragment, the BstEII/BamHI digested PCR  
15 product and long PstI/BamHI V1R-FLpol backbone fragment.

The MRKAd5-gagpol adenovirus vector was constructed using the BglII  
fragment of the V1R-gagpol containing the entire ORF of gag-IAPol fusion gene.

## EXAMPLE 30

## Immunogenicity Studies in Non-Human Primates

20 Cohorts of three (3) macaques were immunized with 10e8 or 10e10 viral  
particles (vp) of one of the following MRKAd5 HIV-1 vaccines: (1) MRKAd5gag;  
(2) MRKAd5pol; (3) MRKAd5nef; (4) a mixture containing equal amounts of  
25 MRKAd5gag, MRKAd5pol, and MRKAd5nef, or (5) a mixture of equal amounts of  
MRKAd5gagpol and MRKAd5nef. The vaccines were administered at weeks 0 and  
4.

The T cell responses against each of the HIV-1 antigens were assayed by IFN-  
gamma ELISpot assay using pools of 20-aa peptides that encompass the entire protein  
30 sequence of each antigen. The results (Table 25) are expressed as the number of spot-  
forming cells (sfc) per million peripheral blood mononuclear cells (PBMC) that  
respond to each of the peptide pools.

Results indicate the following observations: (1) each of the single gene  
constructs (MRKAd5gag, MRKAd5pol, or MRKAd5nef) is able to elicit high levels  
35 of antigen-specific T cells in monkeys; (2) the single-gene MRKAd5 constructs can  
be mixed as a multi-cocktail formulation capable of eliciting very broad T cell  
responses against gag, pol, and nef; (3) the MRKAd5 vector expressing the fusion

protein of gag plus IA pol is capable of inducing strong T cell responses to both gag and pol.

5 **Table 25. Evaluation of Mixtures of MRKAd5 vectors expressing humanized HIV-1 gag, pol, gagpol, nef in rhesus macaques**

Grp #	Vaccine T=0, 4 wks	Monk #	T=6 wks				
			Mock	Gag H	Pol - 1	Pol - 2	Nef
1	MRKAd5 gag 10 <sup>10</sup> vp	CB9V	0	15	-	-	-
		CD19	0	374	-	-	-
		109H	1	843	-	-	-
2	MRKAd5 gag 10 <sup>8</sup> vp	99D130	1	948	-	-	-
		W277	16	324	-	-	-
		143H	4	595	-	-	-
3	MRKAd5 pol 10 <sup>10</sup> vp	CC1X	4	-	46	256	-
		AW3W	3	-	463	550	-
		AV43	6	-	95	1333	-
4	MRKAd5 pol 10 <sup>8</sup> vp	AW38	1	-	19	30	-
		CC8K	0	-	50	995	-
		CC21	1	-	33	436	-
5	MRKAd5 nef 10 <sup>10</sup> vp	076Q	9	-	-	-	1204
		091Q	4	-	-	-	85
		083Q	0	-	-	-	176
6	MRKAd5 nef 10 <sup>8</sup> vp	00C029	1	-	-	-	114
		98D022	6	-	-	-	170
		98D160	3	-	-	-	198
7	MRKAd5gag+MRKAd5pol+MRKAd5nef 10 <sup>10</sup> vp each	99D251	3	206	15	193	120
		05H	3	135	21	9	638
		00C016	3	26	4	51	23
8	MRKAd5gag+MRKAd5pol+MRKAd5nef 10 <sup>8</sup> vp each	99D215	1	171	18	193	240
		81H	5	73	6	14	243
		12H	8	1140	115	811	719
9	MRKAd5gagpol +MRKAd5 nef 10 <sup>10</sup> vp each	99D211	0	83	56	838	725
		22H	4	385	119	1194	1915
		61H	4	343	11	765	853
10	MRKAd5gagpol +MRKAd5 nef 10 <sup>8</sup> vp each	34H	3	78	19	5	75
		48H	1	65	105	46	43
		70H	5	158	15	220	191

Indicated are numbers of spot-forming cells per million PBMCs against the peptide pools. Mock, no peptides; gag H, fifty 20-aa peptides encompassing p55 sequence; pol-1, 20-aa peptides representing N-terminal half of IA pol; pol-2, 20-aa peptides representing the carboxy-terminal half of IA pol; nef, 20-aa peptides encompassing the entire wild-type nef sequence. Responses to the antigens prior to the first immunization did not exceed 40 sfc/10<sup>6</sup> PBMC.

## WHAT IS CLAIMED IS

:

1. A recombinant adenoviral vaccine vector at least partially deleted in  
5 E1 and devoid of E1 activity, comprising:
  - a) an adenovirus *cis*-acting packaging region corresponding to from  
about base pair 1 to between from about base pair 400 to about  
base pair 458 of a wildtype adenovirus genome; and
  - b) a gene encoding an HIV protein or immunologically relevant  
10 modification thereof.
2. A vector in accordance with claim 1 comprising a packaging region  
corresponding to from about base pair 1 to about base pair 450 of a wildtype  
adenovirus genome.
3. A vector in accordance with claim 1 further comprising nucleotides  
15 corresponding to between from about base pair 3511 to about 3524 to about base pair  
5798 of a wildtype adenovirus genome.
4. A vector in accordance with claim 3 comprising base pairs  
corresponding to 1-450 and 3511-5798 of a wildtype adenovirus genome.
5. A vector in accordance with claim 4 which is deleted of base pairs  
20 451-3510.
6. A vector in accordance with claim 1 which is at least partially  
deleted in E3.
7. A vector in accordance with claim 6 wherein the E3 deleted region  
is from base pairs 28,133-30,818.

8. A vector in accordance with claim 1 wherein the gene encoding the HIV protein or modification thereof comprises codons optimized for expression in a human.

9. A vector in accordance with claim 1 wherein the vector comprises a  
5 gene expression cassette comprising:

a) a nucleic acid encoding a protein;

b) a heterologous promoter operatively linked to the nucleic acid encoding the protein; and

(c) a transcription termination sequence.

10 10. A vector in accordance with claim 9 wherein the gene expression cassette is inserted into the E1 region.

11. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette is in an E1 parallel orientation

12. An adenoviral vector in accordance with claim 9 wherein the gene  
15 expression cassette is in an E1 antiparallel orientation.

13. An adenoviral vector in accordance with claim 9 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

14. An adenoviral vector in accordance with claim 13 wherein the promoter is an immediate early human cytomegalovirus promoter.

20 15. An adenoviral vector in accordance with claim 9 wherein the promoter is a murine cytomegalovirus promoter.

16. An adenoviral vector in accordance with claim 9 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

17. An adenoviral vector in accordance with claim 9 wherein the transcription termination sequence is a synthetic polyadenylation signal (SPA).

18. A cell comprising the adenoviral vector of claim 1.

19. Recombinant, replication-defective adenovirus particles harvested  
5 and purified subsequent to transfection of the adenoviral vector of claim 1 into a cell line which expresses adenovirus E1 protein at complementing levels.

20. An HIV vaccine composition comprising purified adenovirus particles of claim 19.

21. An HIV vaccine composition of claim 20 which comprises a  
10 physiologically acceptable carrier.

22. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 1 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant,  
15 replication-defective adenovirus.

23. A method according to claim 22 wherein the cell is a PER.C6<sup>®</sup> cell.

24. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of  
20 claim 21.

25. A method according to claim 24 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

26. A method according to claim 25 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

27. A method according to claim 24 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

28. A method according to claim 24 which comprises administering and readministering the adenovirus vaccine vector to the individual.

29. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV gag or an immunologically relevant modification thereof.

30. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV gag protein or immunologically relevant modification thereof.

31. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:

- a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and
- b) a gene expression cassette comprising
  - i) SEQ ID NO: 29;
  - ii) a heterologous promoter operatively linked to i); and
  - iii) a transcription termination sequence.

32. An adenoviral vector in accordance with claim 31 wherein the gene expression cassette is in an E1 parallel orientation.

33. An adenoviral vector in accordance with claim 31 wherein the gene expression cassette is in an E1 antiparallel orientation.

5           34. An adenoviral vector in accordance with claim 31 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

35. An adenoviral vector in accordance with claim 31 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

10           36. An adenoviral vector in accordance with claim 31 which is at least partially deleted in E3.

37. A cell comprising the adenoviral vector of claim 30.

38. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 30 into a cell  
15 line which expresses adenovirus E1 protein at complementing levels.

39. An HIV vaccine composition comprising purified adenovirus particles of claim 38.

40. An HIV vaccine composition of claim 39 which comprises a physiologically acceptable carrier.

20           41. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 30 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.

42. A method according to claim 41 wherein the cell is a PER.C6<sup>®</sup> cell.

43. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of  
5 claim 21.

44. A method according to claim 43 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

10 45. A method according to claim 44 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

46. A method according to claim 43 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

15 47. A method according to claim 43 which comprises administering and readministering the adenovirus vaccine vector to the individual.

48. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV pol or an immunologically relevant modification thereof.

20 49. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV pol protein or immunologically relevant modification thereof.

50. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:



- 5 a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and
- b) a gene expression cassette comprising
- i) a nucleotide sequence selected the group consisting of SEQ ID NO: 1, SEQ ID NO: 5 and SEQ ID NO: 7;
  - ii) a heterologous promoter operatively linked to i); and
  - 10 iii) a transcription termination sequence.

51. An adenoviral vector in accordance with claim 50 wherein the gene expression cassette is in an E1 parallel orientation.

52. An adenoviral vector in accordance with claim 50 wherein the gene expression cassette is in an E1 antiparallel orientation.

15 53. An adenoviral vector in accordance with claim 50 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

54. An adenoviral vector in accordance with claim 50 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

20 55. An adenoviral vector in accordance with claim 50 which is at least partially deleted in E3.

56. A cell comprising the adenoviral vector of claim 49.

57. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 49 into a cell line which expresses adenovirus E1 protein at complementing levels.

58. An HIV vaccine composition comprising purified adenovirus  
5 particles of claim 57.

59. An HIV vaccine composition of claim 58 which comprises a physiologically acceptable carrier.

60. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of  
10 claim 49 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.

61. A method according to claim 60 wherein the cell is a PER.C6<sup>®</sup> cell.

15 62. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 59.

63. A method according to claim 62 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with  
20 a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

64. A method according to claim 63 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

65. A method according to claim 62 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

66. A method according to claim 62 which comprises administering and readministering the adenovirus vaccine vector to the individual.

5           67. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV nef or an immunologically relevant modification thereof.

68. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV nef protein or immunologically relevant modification thereof.

10           69. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:

a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs  
15           corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and

b) a gene expression cassette comprising

i) a nucleotide sequence selected the group consisting of  
SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13 and  
20           SEQ ID NO: 15;

ii) a heterologous promoter operatively linked to i); and

iii) a transcription termination sequence.

70. An adenoviral vector in accordance with claim 69 wherein the gene expression cassette is in an E1 parallel orientation.

71. An adenoviral vector in accordance with claim 69 wherein the gene expression cassette is in an E1 antiparallel orientation.

72. An adenoviral vector in accordance with claim 69 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

5           73. An adenoviral vector in accordance with claim 69 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

74. An adenoviral vector in accordance with claim 69 which is at least partially deleted in E3.

10           75. A cell comprising the adenoviral vector of claim 68.

76. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 68 into a cell line which expresses adenovirus E1 protein at complementing levels.

15           77. An HIV vaccine composition comprising purified adenovirus particles of claim 76.

78. An HIV vaccine composition of claim 77 which comprises a physiologically acceptable carrier.

79. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 68 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.

20

80. A method according to claim 79 wherein the cell is a PER.C6<sup>®</sup> cell.

81. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 78.

82. A method according to claim 81 which further comprises  
5 administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

83. A method according to claim 82 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus  
10 vaccine.

84. A method according to claim 81 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

85. A method according to claim 81 which comprises administering and readministering the adenovirus vaccine vector to the individual.

15 86. A multivalent adenovirus vaccine composition comprising recombinant, replication-defective adenovirus particles, wherein the adenovirus particles are harvested and purified from a cell line expressing adenovirus E1 protein, and wherein the particles are harvested subsequent to transfection of the cells with an adenoviral vector or vectors in accordance with claim 9; said vector(s) comprising a  
20 gene expression cassette or cassettes comprising nucleotide sequences encoding HIV proteins selected from the group consisting of:

- a) gag, pol, and nef, expressed independently from three individual vectors;

- b) gag, pol, and nef, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
- c) gag, pol, and nef, expressed via two vectors, one expressing a pol-nef fusion, and another expressing gag;
- d) gag, pol, and nef, expressed via two vectors, one expressing a gag-pol fusion and another expressing nef;
- e) gag, pol and nef, expressed via two vectors, one expressing a nef-gag fusion and another expressing pol;
- f) gag, pol, and nef, expressed via one vector expressing a gag-pol-nef fusion;
- g) gag and pol, expressed independently from two individual vectors;
- h) gag and pol, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
- i) pol and nef, expressed independently from two individual vectors;
- j) pol and nef, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
- k) nef and gag, expressed independently from two individual vectors;
- l) nef and gag, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
- m) gag and pol, expressed via one vector expressing a gag-pol fusion;

n) pol and nef, expressed via one vector expressing a pol-nef fusion;  
and

o) nef and gag, expressed via one vector expressing a nef-gag fusion.

87. A multivalent adenovirus vaccine composition in accordance with  
5 claim 86 wherein the gag-pol fusion consists of SEQ ID NO: 39.

88. A multivalent adenovirus vaccine composition in accordance with  
claim 86 wherein the fused sequences have the encoding nucleic acid sequences  
operatively linked to distinct promoters and transcription termination sequences.

89. A multivalent adenovirus vaccine composition in accordance with  
10 claim 86 wherein the fused sequences have the encoding nucleic acid sequences  
operatively linked to a single promoter; and the encoding nucleic acid sequences  
operatively linked by an internal ribosome entry sequence ("IRES").

## Original Adenovector Construct:

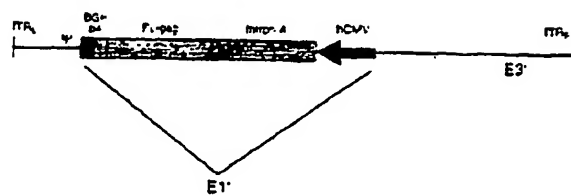


Figure 1: Original HIV-1 gag adenovector.



Sequence of the open reading frame for FL-gag (human codon optimized)

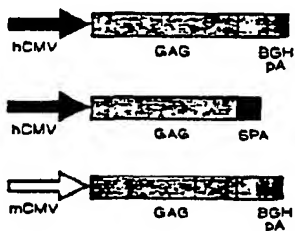
atgggtgctagggcttctgtgctgtctggtggtgagctggacaagtgaggagaagatcaggctgaggccctggg  
caagaagaagtaacaagctaaagcacatgtgtggccctccaggaggctggagaggtttgtgtgaaccctggc  
ctgtgagagacctctgaggggtgcaggcagatccctgggccaagctccagccctccctgcaaacaggctctgagg  
agctgaggtccctgtacaacacagtggttacctgtactgtgtgcaccagaagattgatgtgaaggacaccaag  
gaggccctggagaagattgaggaggagcagaacaagtccaagaagaaggcccagcaggctgtgtgtggc  
acaggcaactccagccagggtgtccagaactaccccatgtgtcagaacctccaggggccagatggtgcaccag  
gccatctccccccggaccctgaatgctgtgggtgaagggtgtggaggagaaggccttctccctgaggtgatccc  
catgttctgtccctgtctgaggggtgccacccccaggaccctgaacaccatgtcgaacacagtggggggccatc  
aggctgccatgcagatgtcgaaggagaccatcaatgaggaggctgtgtgtgtggacaggctgtatcctgtgc  
acgtgtggcccatgtcccccggccagatgaggaggccagggtctgtacatgtcgtggcaccacctccacct  
ccaggagcagatgtgtgtgtgaccaacaaccccccatccctgtgggggaaatctacaagggtgtgatcat  
ccgtggccctgaacaagattgtgaggatgtactccccaccctccatccctggacatcaggcaggggcccaaggag  
cccttcagggaactatgtggacagggttctacaagaccctgagggtgtgagcaggccctccaggagggtgaagaact  
ggatgacagagaccctgtgtgtgtgcagaatgccaaaccctgactgcaagaccatcctgaaggccctgggccctg  
ctgccacccctggaggagatgatgacagccctgccagggtgtggggggccctgtgtcacaaggccagggtgtgtg  
gtgtgaggccatgtcccagggtgaccaactccgccaccatcatgtgtgagaggggcaacttcagggaaccagag  
gaagacagtgaagtgttcaactgtgtgcaagggtgtggccacattgtccaagaactgtaggggcccccagggaaga  
agggctgtgtgaagtgtgtggaaggaggccaccagatgaaggactgtcaatgagaggcaggccaacttctgt  
ggcaaaaatctggccctcccaagaaggcaggcctgtgcaacttctccagtccaggcctgagcccacagccct  
ccgaggagtccttcagggttggggaggagaagaccaccccccagccagaagcaggagcccatgtacaagg  
agctgtacccctgtggccctccctgagggtccctgttggcaacgacccctccctccagtaaaataaagcccgggca  
gat (SEQ ID NO: 29)

Figure 2

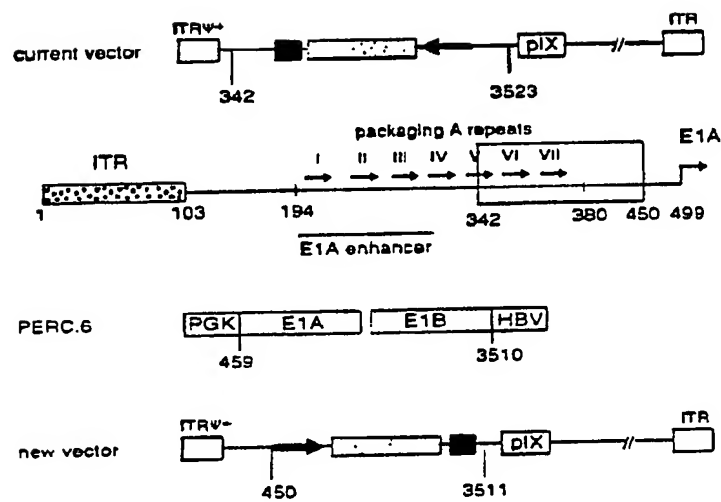
Old Transgene:



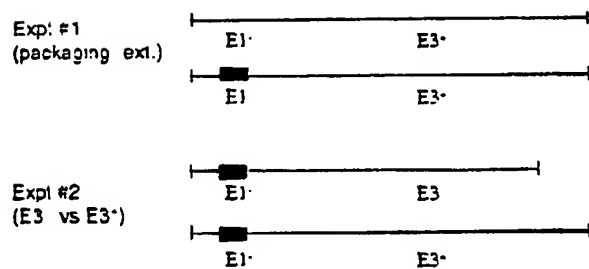
New Transgenes:



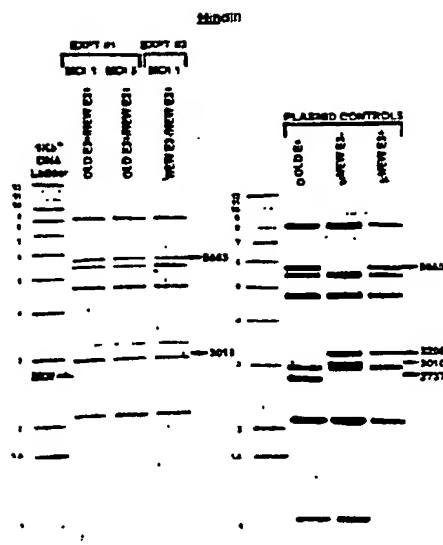
**Figure 3:** Diagrammatic representation of the original HIV-1 gag transgene and the series of new transgene constructions.



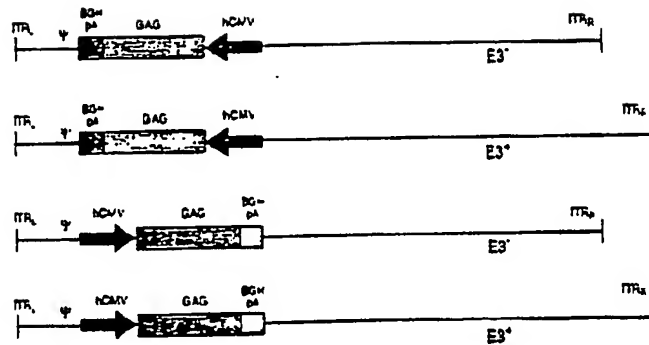
**Figure 4:** Modifications made to the current adenovector backbone in the generation of the new vector.



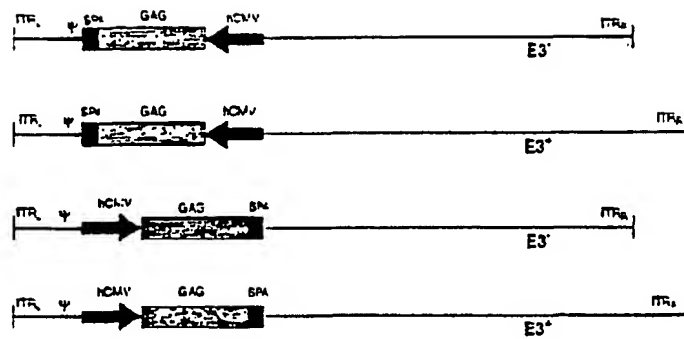
**Figure 5:** Virus mixing experiments to determine the effects of the addition made to the packaging signal region (Expt. #1) and analysis of the effects of the E3 gene on viral growth (Expt. #2). The red bars denote the region of modifications made to the E1 deletion.



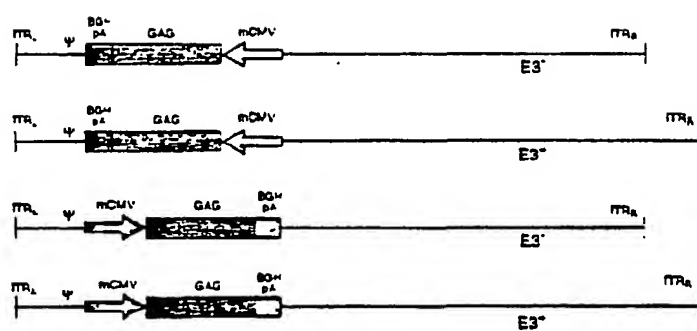
**Figure 6:** Autoradiograph of viral DNA analysis following viral mixing experiments (expts. #1 and #2) as detailed in the text.



**Figure 7A:** hCMV-FLgag-bGHpA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.



**Figure 7B:** hCMV-FLgag-SPA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3<sup>-</sup> and E3<sup>+</sup> backbones were constructed.



**Figure 7C:** mCMV-FLgag-bGHpA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3<sup>-</sup> and E3<sup>+</sup> backbones were constructed.



## Plasmid mixing expt: (orientation)

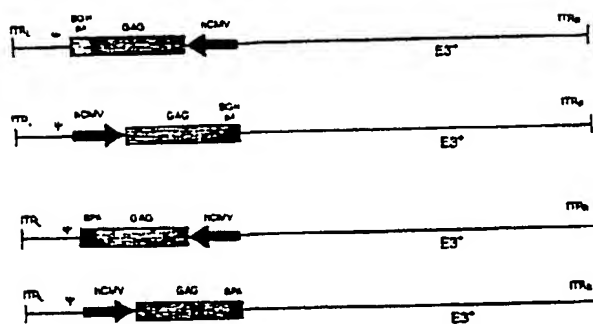


Figure 8A: Effect of transgene orientation

## Plasmid Mixing expt: (poly A signal)

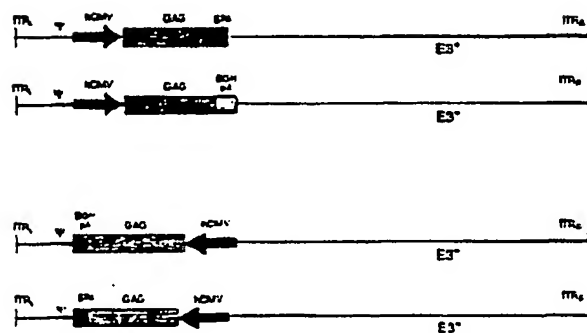


Figure 8B: Effect of polyadenylation signal

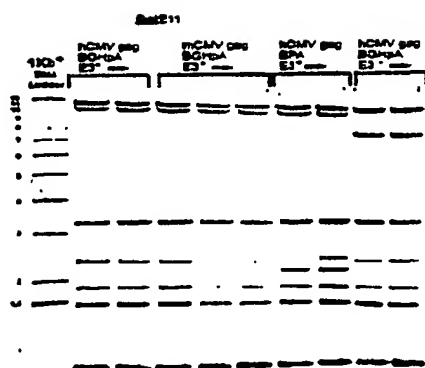


Figure 9: Viral DNA from the four Adgag candidates at P5, following BstE11 digestion.



**Figure 10:** Viral DNA analysis of passage 11 and/or 12 of MRKHVE3, MRKAd5gag and MRKAd5gag(E3-).

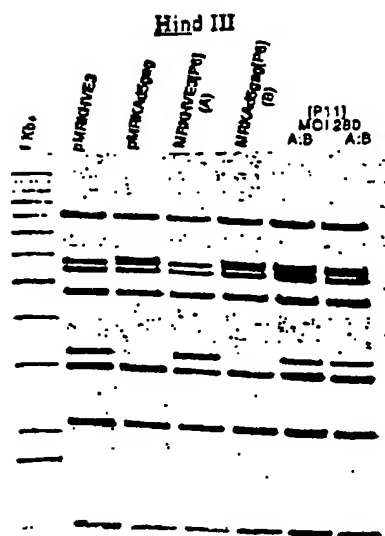
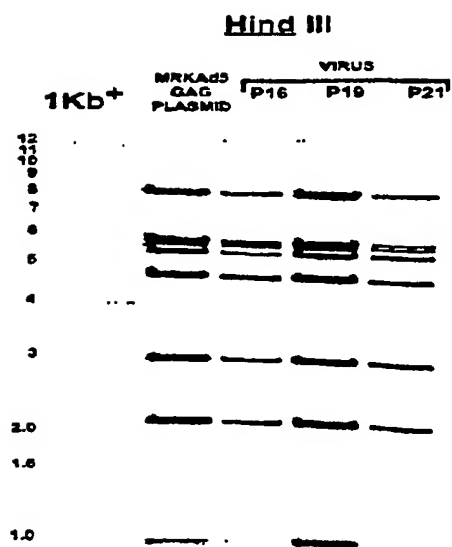
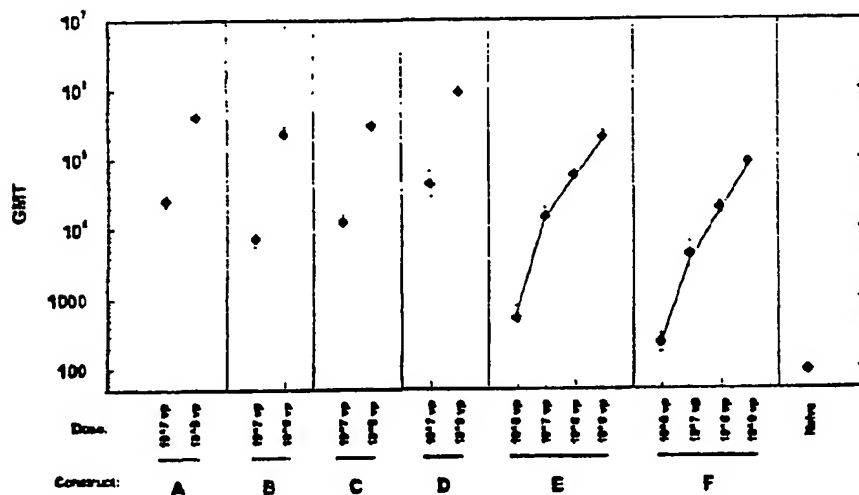


Figure 11: Viral DNA analysis (*Hind*III digestion) of passage 6 MRKHVE3 and MRKAd5gag used to initiate the viral competition study. Last two lanes are passage 11 analysis of duplicate passages of the competition study (each virus at MOI 280 vp).



**Figure 12:** Viral DNA analysis by *Hind*III digestion on high passage numbers for MRKAd5gag in serum containing media with collections made at specified times. The first lane shows the 1 Kb DNA size marker. The other lanes represent pre-plasmid control (digested with *Pac*I and *Hind*III), and MRKAd5gag virus continually passaged to P16, P19 and P21 (serum containing media).

13  
**Figure 13.** Serum anti-p24 Levels at 3 Wks post i.m. immunization of balb/c mice (n=10) with Varying Doses of Several Adgag constructs: (A) MRKAd5gag (through passage 5); (B) MRKAd5 E3<sup>+</sup> hCMV-FLgag-bGHpA; (C) MRKAd5 E3<sup>+</sup> hCMV-FLgag-SPA; (D) MRKAd5 E3<sup>+</sup> mCMV-FLgag-bGHpA; (E) research Lot (293 cell-derived) of Ad5HIV-1gag; and (F) clinical lot (Ad5gagFN0001) of Ad5HIV-1gag. Reported are the geometric mean titers (GMT) for each cohort.



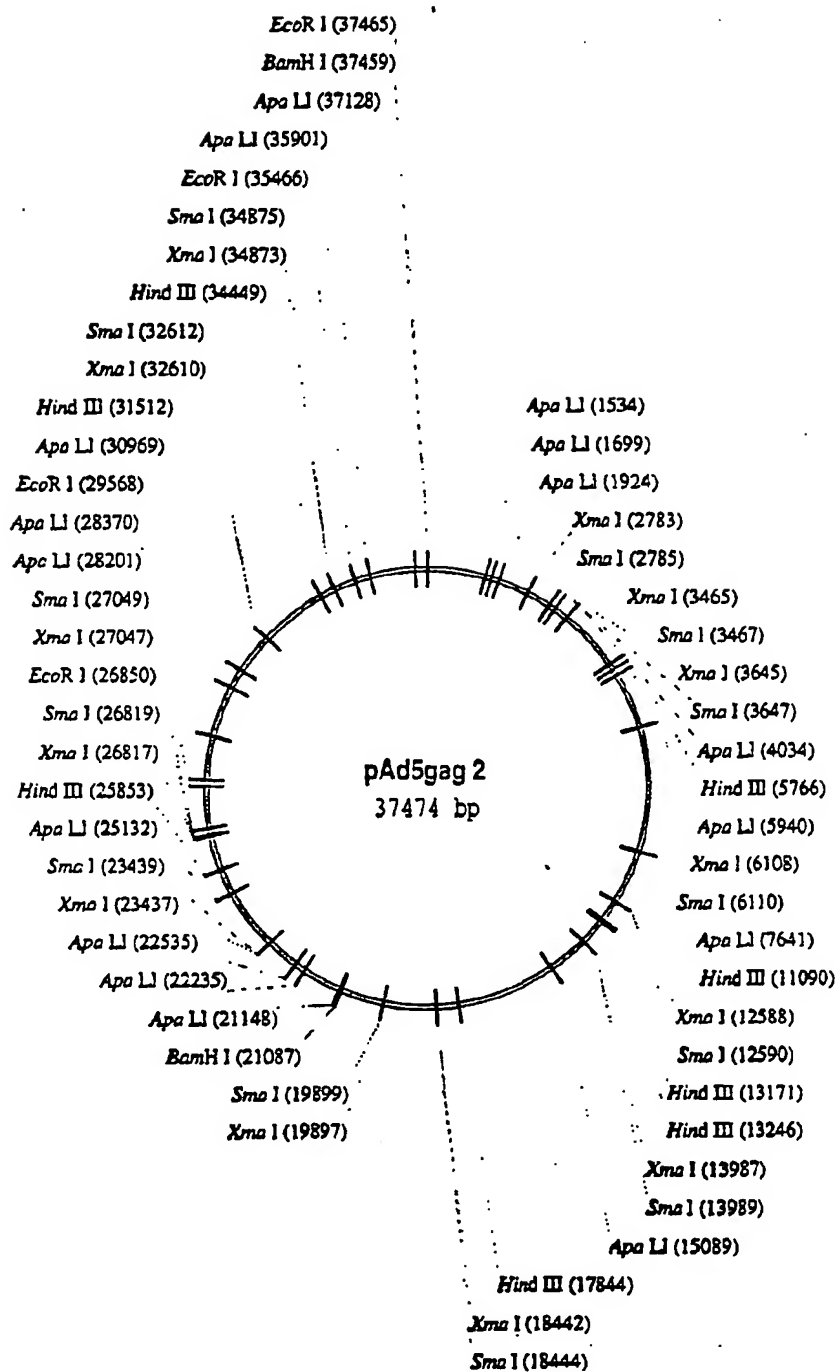


Figure 14



pMRKAd5nag MEU6B2

1	TTCTTAATTA ACATCATCAA TAATATATCT TAATATATCT ATGATTAATTA GGGGATGAG TTATGAGCTT GGGGAGGAG GGGGAGGAG GGGGAGGAG
101	AGCAATTAAT TGTAGTAGTT ATATATATGA ATATATATGA ATATATATGA ATATATATGA ATATATATGA ATATATATGA ATATATATGA ATATATATGA
201	GGGGGAGG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG
301	GGGGGAGG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG
401	GGGGGAGG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG
501	GGGGGAGG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG
601	GGGGGAGG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG
701	GGGGGAGG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG
801	GGGGGAGG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG
901	GGGGGAGG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG
1001	GGGGGAGG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG
1101	GGGGGAGG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG
1201	GGGGGAGG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG
1301	GGGGGAGG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG
1401	GGGGGAGG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG
1501	GGGGGAGG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG
1601	GGGGGAGG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG GAGGAGGAG

Figure 15A

[illegible]

19/144

[illegible]

20/144



## PMRKAd5gag MER6R2

6501 GCGTCACGCA CGAAGGAGGC GTACGAGTGG CCGACCTTGT TTACACGATC GCGCGTGATC TTACAGCTCTA GGGGCGATTA GTCCAGGATT TCCTTCATGA  
 CCGACTGGGT GCTTCCTCCG CATCTTCAGC GATCTGACCA ACTGATGAG AGTGTGAGAT CCGCGTGAT AGGTGCGCA CAGTCCCAA AGCAACTACT  
 TGTGATACTT ATCTGTCCG TTTTCTTCC ATGACTGAGG ATGAGATGAA AATTTTCCG GATCTCTTGG GTACTCTTGG ATCCGAAAC CCGTGTGCTT  
 ACAGTATGAA TAGGACAGGG AAAAAAAGG TGTGAGAGC CAACTCTTGT TTGAGGAGCG CCAAGAGGAT CATGAGAAC TAGCCTTTGG GCAACCTCGA  
 6701 CGAACGGTAA GAGCTTGGCA TGTGAGACTG GTTGTGAGC TTGTAGGAGC AGCATCTCTT TTTTACGCGT AGCGGTATG CCGTCCGCGC CTTCCGCTAG  
 GCTTCCCATT CTGCGATCGT ACATCTTTGAC CAATCTCCCG AGCATCTCTT TLTATAGGAA ATGATGTCTA TCGCGCATAC TCGCGTCCG GAGGCGCTT  
 GAGGTGTGGG TGAGGCGCAA GGTGTCCCTG ACTATGACTT TTGATGATG GTATTTGAG TGTGCTGAG CCGTACGCGG GTGCTCCGAG TCGTTTTTGA  
 CTCACACACC ACTGCGTTT CCACAGGAGC TTGTACTGAA ACTTGCATAC GATTAATCTT TTTCCGCG AGCATACG GACGAGGATC AGGTTTTTGA  
 6901 CCGTCCGCTT TTGTGAGCG GATTTTGGCA GCGGAGGAT GCGATCTTCA CTTATGAC ATGAGTATCT TTTCCGCG AGCATACG TCGCTTTCTT  
 GGCACCGGAA AAGCTTTGG CTTAAACCGT CCGCTTTCCA CTTATGAC ATGAGTATCT TTTCCGCG AGCATACG TCGCTTTCTT  
 7001 TCCCGGACCC AUCCTTCCCA ACATTTATC CTTGAGGAGT GCGGAGGAT GCGATCTTCA CTTATGAC ATGAGTATCT TTTCCGCG AGCATACG TCGCTTTCTT  
 AGGCGCTTGG AUCCTTCCCA ACATTTATC CTTGAGGAGT GCGGAGGAT GCGATCTTCA CTTATGAC ATGAGTATCT TTTCCGCG AGCATACG TCGCTTTCTT  
 7101 GCGATGCGCT TTATGAGAGG CAATTTTGA AGTCTCTGT GCGGAGGAT GCGATCTTCA CTTATGAC ATGAGTATCT TTTCCGCG AGCATACG TCGCTTTCTT  
 CCGTACGCGA ACTACCTTCC GTTAAAGGAT TCAAGGAGC TCTACTGAG TTTCCGCG AGCATACG TTTCCGCG AGCATACG TCGCTTTCTT  
 7201 GGTGAGAGC GAGGATGAG CTTGACAGGT CACTGAGGT TACTGAGT TACTGAGT TACTGAGT TACTGAGT TACTGAGT TACTGAGT TACTGAGT  
 CCAACCTTGG CTGCTTACTC GAGGTGTCCA TTTCCGCGTCA TTTCCGCGTCA TTTCCGCGTCA TTTCCGCGTCA TTTCCGCGTCA TTTCCGCGTCA  
 7301 GGTGAGAGC TCAAGGATGAG GCGGCTTGG TTTCCGCGTCA TTTCCGCGTCA TTTCCGCGTCA TTTCCGCGTCA TTTCCGCGTCA TTTCCGCGTCA  
 CCACTACGTC ATCTTCCAT GCGGCTGAG GCGGCTGAG GCGGCTGAG GCGGCTGAG GCGGCTGAG GCGGCTGAG GCGGCTGAG GCGGCTGAG  
 7401 AACTTCAATG GAGGATGAG GCGGCTGAG GCGGCTGAG GCGGCTGAG GCGGCTGAG GCGGCTGAG GCGGCTGAG GCGGCTGAG GCGGCTGAG  
 TTGATGACT GGTGACTT CCGGCTGAG GCGGCTGAG GCGGCTGAG GCGGCTGAG GCGGCTGAG GCGGCTGAG GCGGCTGAG GCGGCTGAG GCGGCTGAG  
 7501 GATGCGAGCC GATGCGGAG AACTGAGT CCGGCTGAG GCGGCTGAG GCGGCTGAG GCGGCTGAG GCGGCTGAG GCGGCTGAG GCGGCTGAG  
 CTAGGCTGG CTAGGCTGG TTGACCTTGA GGTGCTGG GGTGCTGG GGTGCTGG GGTGCTGG GGTGCTGG GGTGCTGG GGTGCTGG  
 7601 GTGCTGCTT TTGTAAAGC GTGCGAGTA CTGCGAGTG TCGAGCTGG TCGAGCTGG TCGAGCTGG TCGAGCTGG TCGAGCTGG TCGAGCTGG  
 CAGGACCGA AACTTTTTG CACCGCTAT GACGCTGG GACGCTGG GACGCTGG GACGCTGG GACGCTGG GACGCTGG GACGCTGG GACGCTGG  
 7701 GCGAATTTGA GCGGCTGGC TGGGCTTTT GCGTGGTGT GCGTGGTGT GCGTGGTGT GCGTGGTGT GCGTGGTGT GCGTGGTGT GCGTGGTGT  
 CCGTTAACT CCGGAGCGG ACCGCGCAA GCGGCTGG GCGGCTGG GCGGCTGG GCGGCTGG GCGGCTGG GCGGCTGG GCGGCTGG  
 7801 GAGGACCGC GCGGCTGG GCGGCTGG GCGGCTGG GCGGCTGG GCGGCTGG GCGGCTGG GCGGCTGG GCGGCTGG GCGGCTGG  
 CCGGCTGG GCGGCTGG GCGGCTGG GCGGCTGG GCGGCTGG GCGGCTGG GCGGCTGG GCGGCTGG GCGGCTGG GCGGCTGG  
 7901 CTTCCGCGC GTGAGGTCAG GCGGCTGG CTTGAGCTT CTTGAGCTT CTTGAGCTT CTTGAGCTT CTTGAGCTT CTTGAGCTT CTTGAGCTT  
 GAGGCGCGC CAGTCCAGTC CCGGCTGG GCGGCTGG GCGGCTGG GCGGCTGG GCGGCTGG GCGGCTGG GCGGCTGG GCGGCTGG GCGGCTGG  
 8001 TGTGCTGG CCGGCTGG GCGGCTGG GCGGCTGG GCGGCTGG GCGGCTGG GCGGCTGG GCGGCTGG GCGGCTGG GCGGCTGG  
 AAGGACCGC CCGGCTGG GCGGCTGG GCGGCTGG GCGGCTGG GCGGCTGG GCGGCTGG GCGGCTGG GCGGCTGG GCGGCTGG

Figure 15E

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8101	ATGCAATCTAA	AAGCGGTGAC	GCGCGCGACG	CCCGCTGAAAT	AGCTTGAAT	CCCTGACCGC	CCCGAGAGAG	GCGAGGAGCA	CGTGGAGGAC	CGTGGAGGAC	CGTGGAGGAC
8201	TACGTAGATT	TTCGCGACTG	GCGCGCTGAT	GCGCGCTGAT	GCGCGCTGAT	GCGCGCTGAT	GCGCGCTGAT	GCGCGCTGAT	GCGCGCTGAT	GCGCGCTGAT	GCGCGCTGAT
8301	AGAGAGCTGT	CGTGGCGCGG	TAGCTGCTGC	AGCTGCTGCT	AGCTGCTGCT	AGCTGCTGCT	AGCTGCTGCT	AGCTGCTGCT	AGCTGCTGCT	AGCTGCTGCT	AGCTGCTGCT
	TGCTGAGACA	CGAGCGCGCG	ATCCGAGGAC	CGCTGAGGCT	CGCTGAGGCT	CGCTGAGGCT	CGCTGAGGCT	CGCTGAGGCT	CGCTGAGGCT	CGCTGAGGCT	CGCTGAGGCT
8401	CGTGGAGGCT	GAAAGAGAGT	TGCGAGAGAT	CGATGAGGAT	CGATGAGGAT	CGATGAGGAT	CGATGAGGAT	CGATGAGGAT	CGATGAGGAT	CGATGAGGAT	CGATGAGGAT
	CGAACTTGGG	CTTTCTCTCA	AGCTGCTCTA	AGCTGCTCTA	AGCTGCTCTA	AGCTGCTCTA	AGCTGCTCTA	AGCTGCTCTA	AGCTGCTCTA	AGCTGCTCTA	AGCTGCTCTA
8501	GAATCTGCGC	ATGAAGCTGT	CGATCTCTTC	CGATCTCTTC	CGATCTCTTC	CGATCTCTTC	CGATCTCTTC	CGATCTCTTC	CGATCTCTTC	CGATCTCTTC	CGATCTCTTC
	CTAGAGCGCG	TACTTGAGCA	GCTAGAGGAG	GCTAGAGGAG	GCTAGAGGAG	GCTAGAGGAG	GCTAGAGGAG	GCTAGAGGAG	GCTAGAGGAG	GCTAGAGGAG	GCTAGAGGAG
8601	TGCGAGAGAG	CGTTGAGGCG	TGCTGAGGCG	TGCTGAGGCG	TGCTGAGGCG	TGCTGAGGCG	TGCTGAGGCG	TGCTGAGGCG	TGCTGAGGCG	TGCTGAGGCG	TGCTGAGGCG
	AGCTCTTTC	CGAACTTCCG	AGGAGGAGAG	AGGAGGAGAG	AGGAGGAGAG	AGGAGGAGAG	AGGAGGAGAG	AGGAGGAGAG	AGGAGGAGAG	AGGAGGAGAG	AGGAGGAGAG
8701	CGAGGAGGCG	GCGGAGGAG	CGGTAGTTTC	CGGTAGTTTC	CGGTAGTTTC	CGGTAGTTTC	CGGTAGTTTC	CGGTAGTTTC	CGGTAGTTTC	CGGTAGTTTC	CGGTAGTTTC
	CGTGCAGCGC	CGCTCTTTC	CGCATCAAG	CGCATCAAG	CGCATCAAG	CGCATCAAG	CGCATCAAG	CGCATCAAG	CGCATCAAG	CGCATCAAG	CGCATCAAG
8801	TGCGAGGCTG	GAATCTGCTA	TATCCGCTAA	TATCCGCTAA	TATCCGCTAA	TATCCGCTAA	TATCCGCTAA	TATCCGCTAA	TATCCGCTAA	TATCCGCTAA	TATCCGCTAA
	AGCTTTGAC	CTAGAGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT
8901	AGCTTTGAC	CTAGAGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT
	CTAGAGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT
9001	CTAGAGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT
	CTAGAGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT
9101	CTAGAGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT
	CTAGAGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT
9201	CTAGAGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT
	CTAGAGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT
9301	CTAGAGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT
	CTAGAGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT
9401	CTAGAGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT
	CTAGAGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT
9501	CTAGAGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT
	CTAGAGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT
9601	CTAGAGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT
	CTAGAGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT	ATAGCGGCT

Figure 15F

## pMRKad5qag MER82

9701	ACAAAGCGGT GGTATGCGCC CGTGTGATG GTTATATTC AGTACGCTAT AATGAGTAG TTAAGCGTCT GGTACCGCG GACTGCTTCG AGCCACATCG	XbaI
9801	TGAGACGCGA GTAAAGCGTC GAGTCAATA GTTATGCTTT GATACATCTC GTATATCCAC CAATAAGTGC GGGTACCGCT GGGGTATAGC GGGCATCTTC	XbaI
9901	GGGCGACGGT AGGCTGCGCG GGGCTCCGCG GGGGAGATCT TGTAAATATA GGGCATCTAT TGGTATCTA TGGTATCTA TGGTATCTA TGGTATCTA	XbaI
10001	CGGCTGCGCG GGGCTGCGCG GGGCTCCGCG GGGGAGATCT TGTAAATATA GGGCATCTAT TGGTATCTA TGGTATCTA TGGTATCTA TGGTATCTA	XbaI
10101	AAATGTTGAC GCTCTAGACC GTTCAAAAGG GTTCAAAAGG GTTCAAAAGG GTTCAAAAGG GTTCAAAAGG GTTCAAAAGG GTTCAAAAGG GTTCAAAAGG	XbaI
10201	GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC	XbaI
10301	TTTGGCTTCC TTTGGCTTCC TTTGGCTTCC TTTGGCTTCC TTTGGCTTCC TTTGGCTTCC TTTGGCTTCC TTTGGCTTCC TTTGGCTTCC TTTGGCTTCC	XbaI
10401	GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC	XbaI
10501	GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC	XbaI
10601	GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC	XbaI
10701	GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC	XbaI
10801	GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC	XbaI
10901	GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC	XbaI
11001	GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC	XbaI
11101	GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC	XbaI
11201	GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC GGTATGAGC	XbaI

Figure 156

pMRK455gag MER682

11301	TCATTTCAT	AACATCTCG	CAGGATATG	TGTTTACGA	GTTTATTTG	AGCTTACCTG	ACAACTTGT	CGCATCAAC	TATTCCATGC	TTAGCTCTGG
	AGCTAACTA	TTTGTAGGAC	GTTCTGTATC	AGTATTTAC	CTTATTTAC	TTTATTTAC	TTTATTTAC	GGCTTATTTG	ATATGATPAG	ATTCTGACT
11401	CAGATTTCAC	GGCGCAAGA	TATTTATATC	CGTTTATCT	CTTATTTAC	ATATTTATTA	ATATTTATTA	TTCTTATATC	GGATTTTATC	GAATTTTCT
	GTTCATATG	CGGCTTTCT	ATATCTATG	GGGATTTAT	CTTATTTAT	CTTATTTAT	CTTATTTAT	AGATTTTATC	GGATTTTATC	CTTATTTAT
11501	ACTTTGAGG	ACGATCTGG	CTTTTATCG	AGGATTTAT	AGGATTTAT	AGGATTTAT	AGGATTTAT	AGGATTTAT	AGGATTTAT	AGGATTTAT
	TGATTTTCT	TGATTTTCT	TGATTTTCT	TGATTTTCT	TGATTTTCT	TGATTTTCT	TGATTTTCT	TGATTTTCT	TGATTTTCT	TGATTTTCT
11601	GGCTTCATAG	GGCTTCATAG	GGCTTCATAG	GGCTTCATAG	GGCTTCATAG	GGCTTCATAG	GGCTTCATAG	GGCTTCATAG	GGCTTCATAG	GGCTTCATAG
	GGCTTCATAG	GGCTTCATAG	GGCTTCATAG	GGCTTCATAG	GGCTTCATAG	GGCTTCATAG	GGCTTCATAG	GGCTTCATAG	GGCTTCATAG	GGCTTCATAG
11701	CTTGTAGGCA	CTTGTAGGCA	CTTGTAGGCA	CTTGTAGGCA	CTTGTAGGCA	CTTGTAGGCA	CTTGTAGGCA	CTTGTAGGCA	CTTGTAGGCA	CTTGTAGGCA
	CTTGTAGGCA	CTTGTAGGCA	CTTGTAGGCA	CTTGTAGGCA	CTTGTAGGCA	CTTGTAGGCA	CTTGTAGGCA	CTTGTAGGCA	CTTGTAGGCA	CTTGTAGGCA
11801	CGATAGGAG	CGATAGGAG	CGATAGGAG	CGATAGGAG	CGATAGGAG	CGATAGGAG	CGATAGGAG	CGATAGGAG	CGATAGGAG	CGATAGGAG
	CGATAGGAG	CGATAGGAG	CGATAGGAG	CGATAGGAG	CGATAGGAG	CGATAGGAG	CGATAGGAG	CGATAGGAG	CGATAGGAG	CGATAGGAG
11901	CTTAACTCC	ACGATGACT	GGGCTGAGT	CTTAACTCC	CTTAACTCC	CTTAACTCC	CTTAACTCC	CTTAACTCC	CTTAACTCC	CTTAACTCC
	CTTAACTCC	ACGATGACT	GGGCTGAGT	CTTAACTCC	CTTAACTCC	CTTAACTCC	CTTAACTCC	CTTAACTCC	CTTAACTCC	CTTAACTCC
12001	CTCTTCGCA	TTCTTGAGC	CTCTTCGCA	TTCTTGAGC	CTCTTCGCA	TTCTTGAGC	CTCTTCGCA	TTCTTGAGC	CTCTTCGCA	TTCTTGAGC
	CTCTTCGCA	TTCTTGAGC	CTCTTCGCA	TTCTTGAGC	CTCTTCGCA	TTCTTGAGC	CTCTTCGCA	TTCTTGAGC	CTCTTCGCA	TTCTTGAGC
12101	GGCTTCGCA	GGCTTCGCA	GGCTTCGCA	GGCTTCGCA	GGCTTCGCA	GGCTTCGCA	GGCTTCGCA	GGCTTCGCA	GGCTTCGCA	GGCTTCGCA
	GGCTTCGCA	GGCTTCGCA	GGCTTCGCA	GGCTTCGCA	GGCTTCGCA	GGCTTCGCA	GGCTTCGCA	GGCTTCGCA	GGCTTCGCA	GGCTTCGCA
12201	TTGTGGGAG	GGCTTCGCA	GGCTTCGCA	GGCTTCGCA	GGCTTCGCA	GGCTTCGCA	GGCTTCGCA	GGCTTCGCA	GGCTTCGCA	GGCTTCGCA
	TTGTGGGAG	GGCTTCGCA	GGCTTCGCA	GGCTTCGCA	GGCTTCGCA	GGCTTCGCA	GGCTTCGCA	GGCTTCGCA	GGCTTCGCA	GGCTTCGCA
12301	GTGCTTCGCA	GTGCTTCGCA	GTGCTTCGCA	GTGCTTCGCA	GTGCTTCGCA	GTGCTTCGCA	GTGCTTCGCA	GTGCTTCGCA	GTGCTTCGCA	GTGCTTCGCA
	GTGCTTCGCA	GTGCTTCGCA	GTGCTTCGCA	GTGCTTCGCA	GTGCTTCGCA	GTGCTTCGCA	GTGCTTCGCA	GTGCTTCGCA	GTGCTTCGCA	GTGCTTCGCA
12401	ATTTTTCGA	ATTTTTCGA	ATTTTTCGA	ATTTTTCGA	ATTTTTCGA	ATTTTTCGA	ATTTTTCGA	ATTTTTCGA	ATTTTTCGA	ATTTTTCGA
	ATTTTTCGA	ATTTTTCGA	ATTTTTCGA	ATTTTTCGA	ATTTTTCGA	ATTTTTCGA	ATTTTTCGA	ATTTTTCGA	ATTTTTCGA	ATTTTTCGA
12501	GGCTTCGCA	GGCTTCGCA	GGCTTCGCA	GGCTTCGCA	GGCTTCGCA	GGCTTCGCA	GGCTTCGCA	GGCTTCGCA	GGCTTCGCA	GGCTTCGCA
	GGCTTCGCA	GGCTTCGCA	GGCTTCGCA	GGCTTCGCA	GGCTTCGCA	GGCTTCGCA	GGCTTCGCA	GGCTTCGCA	GGCTTCGCA	GGCTTCGCA
12601	CTAGTTCACT	CTAGTTCACT	CTAGTTCACT	CTAGTTCACT	CTAGTTCACT	CTAGTTCACT	CTAGTTCACT	CTAGTTCACT	CTAGTTCACT	CTAGTTCACT
	CTAGTTCACT	CTAGTTCACT	CTAGTTCACT	CTAGTTCACT	CTAGTTCACT	CTAGTTCACT	CTAGTTCACT	CTAGTTCACT	CTAGTTCACT	CTAGTTCACT
12701	AGGATGAGC	AGGATGAGC	AGGATGAGC	AGGATGAGC	AGGATGAGC	AGGATGAGC	AGGATGAGC	AGGATGAGC	AGGATGAGC	AGGATGAGC
	AGGATGAGC	AGGATGAGC	AGGATGAGC	AGGATGAGC	AGGATGAGC	AGGATGAGC	AGGATGAGC	AGGATGAGC	AGGATGAGC	AGGATGAGC
12801	CAATTTTCC	CAATTTTCC	CAATTTTCC	CAATTTTCC	CAATTTTCC	CAATTTTCC	CAATTTTCC	CAATTTTCC	CAATTTTCC	CAATTTTCC
	CAATTTTCC	CAATTTTCC	CAATTTTCC	CAATTTTCC	CAATTTTCC	CAATTTTCC	CAATTTTCC	CAATTTTCC	CAATTTTCC	CAATTTTCC

Figure 15H



## pMRKad5qng MER62

12901 GGCATGATG GCTCAAAAGG GCGCTTTATC AACCTGCTTA TTAATTAATCT GATTTATCTG GTGCTGCTTA ACCCGAGTA TTTCACCAAT GCTATCTTGA  
 CCGTACATAC GGAGTTTGG CCGCAATATG TTGATTAAT ACCTATATCA CTTATGATAC GATGAGCAT TTAGAGTCAAT AATAGTGTTA AATAGTCAAT CXTATCAACT  
 13001 ACCCGACTG GCTACCGCC CCGCTTTTCT ACACCTGATG ATTCAATGATG CTTGATGATG AATATGATG CTTATGAGGAC GACTATAGCG ACGATGCTTT  
 TGGCGTGAC CGATGCGCG GACCAAAAGA TTATGCTGCG TAACTATAC ATGTTCTCAT TCTATCTTGA GAGACCGCTG CTTATATGCG TGTGCGCAAA  
 13101 TTCCCGGGA CCGCAGACC TCGTATGATG GTATACATG GATGAGGAC AGCGTGTCT GTTAAAGGAA AGCTTCCGA GCGCAGCAG CTTGTGCGGT  
 AAGCGCGCTT GCGCTCTGCG ACATCTCAA CTTGTGCGG CTTGATGATG TCTGCTGCTA CTTCTCTT TCGAAGCGCT CCGTATCTG GACAGGCTT  
 13201 CTAGGCGTG CCGCGCGCG GTACATGCT AGTATGCTT TTTCAATGCT CATAGGCTCT CTACAGCA CTTCCAGCAC CCGCGCGCG CTTGCTGCG  
 GATCCCGAC GCGCGCGCG CAGTCTAGCA TCTATGCTA AGCTTCTGA CTATCCAGA GATGCTGT GAGCTGCTG GCGCGCGCG GACCGCGCG  
 13301 AAGGAGGTA CTTAAACAC TCGTCTGCTG AGCGCGCG GGAAMAMAC CTTGCTGCTG CATTTCTGA CAGAGGATA GAGAGCTG TCGACATAT  
 TCTCTCTCAT GATTTCTGTT AGCGAGCG TCGCTGCTG CTTTCTGCTG GATGAGGCT GTTACGCTAT CTTCTGCTAT CTTCTGCTAT ACCTTCTCTA  
 13401 GATGATATG AGACGTTAG CCGAGGAGTA CAGGAGCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG  
 CTTATCTAC TTCTGCTG CCGCTCTGCT GTCTCTGCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG  
 13501 GAGGAGGTA ACTGCGAG CAGACGAC CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG  
 CTTCTCTAC TTAGGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG  
 13601 AAAAAAA CTTATGATG AATATATTA CTTACCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG  
 TTTTCTCTT CTTATCTCT TTTATCTCT GATGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG  
 13701 TTAGGAGGCT CTTCTCTCT CTTACGAGG TGTGCTGCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG  
 ACTCTCTTCCA GAGAGGGA GATCTCTC ACACCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG  
 13801 GTGCTCTG GATCTCTG GATCTCTG GATCTCTG GATCTCTG GATCTCTG GATCTCTG GATCTCTG GATCTCTG GATCTCTG GATCTCTG  
 CAGGAGGCT CAGGAGGCT CAGGAGGCT CAGGAGGCT CAGGAGGCT CAGGAGGCT CAGGAGGCT CAGGAGGCT CAGGAGGCT CAGGAGGCT CAGGAGGCT  
 13901 ACAGGCTAC GATGCTGCTA TCGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG  
 TTTTCTCTG CTTATCTCT TTTATCTCT GATGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG  
 14001 CAGCAGGCT ATCACTCTG AGGAGGCT GATGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG  
 GTGCTCTG TATGCTCTG TATGCTCTG TATGCTCTG TATGCTCTG TATGCTCTG TATGCTCTG TATGCTCTG TATGCTCTG TATGCTCTG TATGCTCTG  
 14101 AATAGGTTA AGGAGGCT GATGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG  
 TATGCTCTG TATGCTCTG TATGCTCTG TATGCTCTG TATGCTCTG TATGCTCTG TATGCTCTG TATGCTCTG TATGCTCTG TATGCTCTG  
 14201 ACTACTCTG GATGCTCTG GATGCTCTG GATGCTCTG GATGCTCTG GATGCTCTG GATGCTCTG GATGCTCTG GATGCTCTG GATGCTCTG  
 TATGCTCTG TATGCTCTG TATGCTCTG TATGCTCTG TATGCTCTG TATGCTCTG TATGCTCTG TATGCTCTG TATGCTCTG TATGCTCTG  
 14301 GATGCTCTG GATGCTCTG GATGCTCTG GATGCTCTG GATGCTCTG GATGCTCTG GATGCTCTG GATGCTCTG GATGCTCTG GATGCTCTG  
 CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG CAGCTCTG  
 14401 AATGCTCTG GATGCTCTG GATGCTCTG GATGCTCTG GATGCTCTG GATGCTCTG GATGCTCTG GATGCTCTG GATGCTCTG GATGCTCTG  
 TATGCTCTG TATGCTCTG TATGCTCTG TATGCTCTG TATGCTCTG TATGCTCTG TATGCTCTG TATGCTCTG TATGCTCTG TATGCTCTG

Figure 15I

## pMIRAd5seq HER6R2

14501 CTTACGATTA TCTGAGGCT GTTACATTC CCGACATCT GTATACAGT GTATACAGT AGATACAGT CAGACAGGCT GCGCTATCTC  
 GGTATCTACT AGACCTCTCA CCAATGTAA GCGGTACCA CCAATGTAA CCAATGTAA CCAATGTAA CCAATGTAA CCAATGTAA  
 14601 AGGCGCAGC AACAGCAGT GCAATCTCT GCAATCTCT GCAATCTCT GCAATCTCT GCAATCTCT GCAATCTCT GCAATCTCT  
 TCCCGCTCG TTGCTGTCG CTTCTCTCT AGCTCTCT AGCTCTCT AGCTCTCT AGCTCTCT AGCTCTCT AGCTCTCT  
 14701 GCGCAGCTT TTGCGACAG GCGTACAGG AGCTCTCT AGCTCTCT AGCTCTCT AGCTCTCT AGCTCTCT AGCTCTCT  
 CCGCTGTGA AACGCTGTC CCGACCTCT TTGCGCTCT TTGCGCTCT TTGCGCTCT TTGCGCTCT TTGCGCTCT TTGCGCTCT  
 14801 AGAGGAAAC GGTGATCAA CCGCTACAG AATACAGT TACACAGT TACACAGT TACACAGT TACACAGT TACACAGT  
 TCTCTTTTG CCACTAGTTT GCGGACTGT TCTCTCTCT TCTCTCTCT TCTCTCTCT TCTCTCTCT TCTCTCTCT TCTCTCTCT  
 14901 CTTTGCATAC AACTAGGCG ACCCTACAG CCGATCTCT TCTCTCTCT TCTCTCTCT TCTCTCTCT TCTCTCTCT TCTCTCTCT  
 GGAAGCTAT TTGATGCGC TTGATGCGC TTGATGCGC TTGATGCGC TTGATGCGC TTGATGCGC TTGATGCGC TTGATGCGC  
 15001 TTGCGAGCA TGAATGAGA CCGCTGAGC CCGCTGAGC CCGCTGAGC CCGCTGAGC CCGCTGAGC CCGCTGAGC CCGCTGAGC  
 AACGCTCTT ACTAGCTTT AGGCGCTCT AGGCGCTCT AGGCGCTCT AGGCGCTCT AGGCGCTCT AGGCGCTCT AGGCGCTCT  
 15101 GCTTCTTACA CCGACAGGC GTCTACTTC AACTCATCT CCGCTTCTC TCTCTCTCT AACTCTCTC AACTCTCTC AACTCTCTC  
 CCGAGATGTT CCGCTCTCT CCGAGATGTT CCGAGATGTT CCGAGATGTT CCGAGATGTT CCGAGATGTT CCGAGATGTT CCGAGATGTT  
 15201 CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT  
 GCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT  
 15301 GTGACCATTA CTTACGCTAG ACCCTCTCT TTGACAGCT CTTACAGCT CTTACAGCT CTTACAGCT CTTACAGCT CTTACAGCT  
 CACTGCTTAT GACTGCTCT TCGCGCTCT ACCGCTCT ACCGCTCT ACCGCTCT ACCGCTCT ACCGCTCT ACCGCTCT ACCGCTCT  
 15401 GCAATGCTAT CTTATATCT CCGAGATTA CCGAGATTA CCGAGATTA CCGAGATTA CCGAGATTA CCGAGATTA CCGAGATTA  
 CCGAGATTA CCGAGATTA CCGAGATTA CCGAGATTA CCGAGATTA CCGAGATTA CCGAGATTA CCGAGATTA CCGAGATTA  
 15501 AGTCTGCTG CCGCTCTCT ACCGCTCT ACCGCTCT ACCGCTCT ACCGCTCT ACCGCTCT ACCGCTCT ACCGCTCT ACCGCTCT  
 TCACGCTAC GCGCTCTCT TCGCGCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT  
 15601 GAGCGCTCA ACTACAGCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT  
 CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT  
 15701 GAGCGCTCA GCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT  
 CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT  
 15801 AGGCGCTCA ATGCGCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT  
 TCGCGCTCA TCGCGCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT  
 15901 AGTCTCTGA CTTACGCTG CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT  
 TCACGCTAT GACTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT  
 16001 TTGCGAGAA AACCTCTTA GACTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT  
 AACCTCTTT TTGCGAGAA CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT CCGCTCTCT

Figure 15J



[illegible]

Figure 15L

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19301	AGACATATATG GGCACACAT CTATATCCAA CAGCTTAT TACATTTGTT TTATATATAT TTTATATATG CTATATGATTT ACACAGGCAC GGTATATATG GGTATATATG
19401	TCTTGATATAC CGGTGATTTA GATACGGTGT GTCTATATTA ATTTATACAA ATATCTGTTT AAATATATCA GATTACATAA GATTACATAA TGTATATATG
19501	GGTGATCTGG CGGACCAAC ATCGAGTTT TAGATTTGCA ACACAGAAC ACAGACTTTT CATACAGCTT CATACAGCTT TTTGCTTGAT TCCATATCTG
19601	CCACAGACC GCGGATTCG TAGGCTCAAC ATTCATATCTT TAGATTTGCA ACACAGAAC ATTCATATCTT TGTCTGAA TGTCTGAA TGTCTGAA TGTCTGAA
19701	ATAGAACAG GTACTTTTCT ATCTATATC AGCTTTTCA CAGCTATGAT CCAGATTTTA GATATATGTA TTTATATGTA TTTATATGTA TTTATATGTA TTTATATGTA
19801	TATCTTGCTT CCACTGGAG GTCTATTTA TACAGAGCT TTATATATC TAAATCTAA ATATATATC ATATATATC ATATATATC ATATATATC ATATATATC
19901	AAATGCTAT TTTACTTTA TTTCTAACCT TATATATATC TTTATATATC TTTATATATC TTTATATATC TTTATATATC TTTATATATC TTTATATATC TTTATATATC
20001	TTTCTGATA AAATGCTAT TTTCTAACCT TATATATATC TTTATATATC TTTATATATC TTTATATATC TTTATATATC TTTATATATC TTTATATATC TTTATATATC
20101	TTTCTGATA AAATGCTAT TTTCTAACCT TATATATATC TTTATATATC TTTATATATC TTTATATATC TTTATATATC TTTATATATC TTTATATATC TTTATATATC
20201	TTTCTGATA AAATGCTAT TTTCTAACCT TATATATATC TTTATATATC TTTATATATC TTTATATATC TTTATATATC TTTATATATC TTTATATATC TTTATATATC
20301	TTTCTGATA AAATGCTAT TTTCTAACCT TATATATATC TTTATATATC TTTATATATC TTTATATATC TTTATATATC TTTATATATC TTTATATATC TTTATATATC
20401	TTTCTGATA AAATGCTAT TTTCTAACCT TATATATATC TTTATATATC TTTATATATC TTTATATATC TTTATATATC TTTATATATC TTTATATATC TTTATATATC
20501	TTTCTGATA AAATGCTAT TTTCTAACCT TATATATATC TTTATATATC TTTATATATC TTTATATATC TTTATATATC TTTATATATC TTTATATATC TTTATATATC
20601	TTTCTGATA AAATGCTAT TTTCTAACCT TATATATATC TTTATATATC TTTATATATC TTTATATATC TTTATATATC TTTATATATC TTTATATATC TTTATATATC
20701	TTTCTGATA AAATGCTAT TTTCTAACCT TATATATATC TTTATATATC TTTATATATC TTTATATATC TTTATATATC TTTATATATC TTTATATATC TTTATATATC
20801	TTTCTGATA AAATGCTAT TTTCTAACCT TATATATATC TTTATATATC TTTATATATC TTTATATATC TTTATATATC TTTATATATC TTTATATATC TTTATATATC
20901	TTTCTGATA AAATGCTAT TTTCTAACCT TATATATATC TTTATATATC TTTATATATC TTTATATATC TTTATATATC TTTATATATC TTTATATATC TTTATATATC

Figure 15M

[illegible]

Figure 15N

[illegible]

Figure 15D



## pMRKΔ5gag MER6R2

24201 CTTGCTGCAA CGAAGTCCCA AAAATCTTTG AGCTGACGAG AATGCTCTG CAAAGCTCTT GACACAGGAA AACAGGCCAA ATGAAAGCTA  
 GAGGCCAGTT GCTTCAGGCT TTTTAGAATC TCCAGAAAC TGGCTCTCTC TTGCTGCTCC GTTGTGTGAA GTTGTGCTTT TTGTGCTCTT TACTTTCTAGT  
 ~~~~~  
 24301 CTCTGCGATG TTGCTGCAAC TGTAGGCTGA CAAAGCTCTC CTAGCTCTAC TAAATCTAG CATGAGCTC ACCACTTTG CTTACCCGCG ACTTAACCTA  
 GAGACTCAC AACACCTTTG AGCTCCACT GTTCTGCTCG GATTCGATG AATTCTCTCG GTAGCTCAG TATGCTGAAC GATGCGCG TGAAATTTGAT TGAATTTGAT  
 24401 CCCCCCAAGG TCAATGAGAC AGTCATAGCT GATCTAGCTG TGTGCTGATG TGTGCTGATG TGTGCTGATG TGTGCTGATG TGTGCTGATG TGTGCTGATG  
 GGGGGTTCC AGTACTCTG TCACTACTCA CACTACTGAC ACCTGCTGAC ACCTGCTGAC ACCTGCTGAC ACCTGCTGAC ACCTGCTGAC ACCTGCTGAC  
 24501 TACCGGAGT TGGCGAGAG CACTAGTC CACTAGTC CACTAGTC CACTAGTC CACTAGTC CACTAGTC CACTAGTC CACTAGTC  
 ATGGGCTCA ACCGCTCTC GTGATCTG CGACCTGAG TTGCTGCTC GACGCTGAC ACCTGCTGAC ACCTGCTGAC ACCTGCTGAC ACCTGCTGAC  
 ~~~~~  
 24601 TACGCTGAG CTTGCTGCA TGCAGCGTT CTTGCTGAC CCGCTGATC AGCTGATC AGCTGATC AGCTGATC AGCTGATC AGCTGATC  
 ATGCGACTC GAGCTGAGT ACCTGCTCA ACCTGCTCA GAGCTGATC GAGCTGATC GAGCTGATC GAGCTGATC GAGCTGATC GAGCTGATC  
 ~~~~~  
 24701 CCGCAGGCT GCAAGATCTC CAAGCTGAG CTTGCTGAC TGTCTCTC CTTGCTGAT TGTCTCTC CTTGCTGAT TGTCTCTC CTTGCTGAT  
 GCGCTCCGCA CTTCTGAG GTTGCTGAT GAGCTGATC GAGCTGATC GAGCTGATC GAGCTGATC GAGCTGATC GAGCTGATC GAGCTGATC  
 ~~~~~  
 24801 CCGCTAAGG CAGGCGCG CCGCTGATC TCGCTGATC CTTGCTGAT TGTCTCTC CTTGCTGAT TGTCTCTC CTTGCTGAT TGTCTCTC  
 GCGAGTTCCC GCTGCTGCG GCGCTGATC GCGCTGATC GCGCTGATC GCGCTGATC GCGCTGATC GCGCTGATC GCGCTGATC GCGCTGATC  
 ~~~~~  
 24901 GAGGAGTGC AAGCTGAGG AGCTGAGCA ACTGCTAAG CAAACTTGA AGCTGATG GAGCTGATC GAGCTGATC GAGCTGATC GAGCTGATC  
 CTTCTGAGG TTGAGTTCC TCGAGTTCT TCACTATTC GTTCTGAT TGTCTCTC CTTGCTGAT TGTCTCTC CTTGCTGAT TGTCTCTC  
 25001 GACATCATTT TCGCGAAG CTTGCTTAA ACCCTGAC AGCTGATC AGCTGATC AGCTGATC AGCTGATC AGCTGATC AGCTGATC  
 CTGTAGTAA AGGCTTTC GAGCTGAT TCGAGCTG TCGAGCTG TCGAGCTG TCGAGCTG TCGAGCTG TCGAGCTG TCGAGCTG  
 25101 AGGCTGAGG AATCTTCCC GCGCTGAT CTTGCTGAT CTTGCTGAT CTTGCTGAT CTTGCTGAT CTTGCTGAT CTTGCTGAT CTTGCTGAT  
 TCGGAGTCC TTGAGACCG CCGCTGAG CAGCTGAG CAGCTGAG CAGCTGAG CAGCTGAG CAGCTGAG CAGCTGAG CAGCTGAG  
 ~~~~~  
 25201 CTTGCTGAG CTAGGCACT ACCTGCTCA CCACTGAT CCACTGAT CCACTGAT CCACTGAT CCACTGAT CCACTGAT CCACTGAT  
 GAGAGAGTC GATGCTTGA TGGAGCGAT GGTGAGACT GTTACCTTC TGTACCTTC TGTACCTTC TGTACCTTC TGTACCTTC TGTACCTTC  
 ~~~~~  
 25301 ACCCGGACC GCTGCTGAT TTGCAATTC CAGCTGCTA AGCAAGTCA AATTAAGTT ACCTTCTG CAGCTGCTA AATTAAGTT ACCTTCTG CAGCTGCTA  
 TGGGCTGAG CAGGAGGACA AAGCTTAAAG GTTCTGAGT TGTCTGAT TGTCTGAT TGTCTGAT TGTCTGAT TGTCTGAT TGTCTGAT  
 25401 CCGCTGCTGAG GTTGTAAATC ACTCGCGCG TGTGAGCTC GCTTACCTT TGTCTGAT TGTCTGAT TGTCTGAT TGTCTGAT TGTCTGAT  
 GCGAGGCGC CAACTTTGAG TGGGCTGAG ACCTGCTG CAGCTGAG CAGCTGAG CAGCTGAG CAGCTGAG CAGCTGAG CAGCTGAG  
 25501 AGACCAATCC CCGCGCTCA ATCGGAGCT TACGCTGAG TGTCTGAT TGTCTGAT TGTCTGAT TGTCTGAT TGTCTGAT TGTCTGAT  
 TGTCTGAT TGTCTGAT TGTCTGAT TGTCTGAT TGTCTGAT TGTCTGAT TGTCTGAT TGTCTGAT TGTCTGAT TGTCTGAT  
 25601 TTTCTCTAC GAGAGGAGT GGGGTTTTAC TTGAGCTGAG CAGCTGATC CAGCTGATC CAGCTGATC CAGCTGATC CAGCTGATC CAGCTGATC  
 AAGAGGATG CTTTCCCTG CCCCCAAATG AACTGCTGAG AACTGCTGAG AACTGCTGAG AACTGCTGAG AACTGCTGAG AACTGCTGAG AACTGCTGAG

Figure 1SP



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|       |           |           |           |           |           |           |           |           |           |           |          |
|-------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|----------|
| 25701 | GGCCCTTTC | TTCCACGAT | GGACGCGAA | AACAACTTC | ATTTATTTT | CTTACCTTC | TAACGAGAT | ATATCTGCA | CAATCAGCA | GATGAGTTT | CCCTCCCA |
|       | CCCGGAGAG | AGGGTCTTA | CCGTGTTT  | TTCTTCGAG | TTCAAGAGG | CTTTATGAT | CTTCTGCTC | TTATGACCT | GTACAGTCT | CTCTCCCA  |          |
|       |           |           |           |           |           |           |           |           |           |           |          |
| 25801 | TTGACGAGA | GGAGAGGAC | ATGATCGAG | ACTTGGAG  | CTTATGAG  | GAAGCTTTC | GAAGCTTTC | GGTGTGAG  | GAAGACCTT | CACTCTTC  |          |
|       | ACCTGCTCT | CTCTCTCTC | TACTTCTTC | TGACCTCTC | GGATCTCTC | CTTCTGAGC | TCCAGCTCT | CCACAGTCT | CTTCTGAGC | GTGAGCTTA |          |
| 25901 | CGCATTTCC | TCGCGCGGC | CCAGAAATC | GGCAAGCTT | TCAGATATG | CTACAACTC | CTCTCTCTC | GGCGCGCGC | CAATGCGCT | TCAGCGAGC |          |
|       | CGCTAAAGG | AGCGCGCGC | GGTCTTTAG | CGCTTTTTC | AGCTTCTTC | GGTCTTTC  | GGTCTTTC  | GGTCTTTC  | GGTCTTTC  | GGTCTTTC  |          |
| 26001 | AACCTTATG | GGGACACAC | TCGAAACAG | GGCTTATTA | GGCTTATTA | GGCTTATTA | GGCTTATTA | GGCTTATTA | GGCTTATTA | GGCTTATTA |          |
|       | TTGCACTTA | CCCTGCTTC | ACCTGCTTC | GGCTTATTA | GGCTTATTA | GGCTTATTA | GGCTTATTA | GGCTTATTA | GGCTTATTA | GGCTTATTA |          |
| 26101 | CGCGGCGAA | GAAGGCTTA | GGTCTTCTC | GGTCTTCTC | GGTCTTCTC | GGTCTTCTC | GGTCTTCTC | GGTCTTCTC | GGTCTTCTC | GGTCTTCTC |          |
|       | CGCGGCTTT | CTTCTCTAT | CAAGCAACA | AGCTTCTAT | AGCTTCTAT | AGCTTCTAT | AGCTTCTAT | AGCTTCTAT | AGCTTCTAT | AGCTTCTAT |          |
| 26201 | CGCTTATAT | CTGCTTATC | ACCTTATCT | CTGCTTATC | CTGCTTATC | CTGCTTATC | CTGCTTATC | CTGCTTATC | CTGCTTATC | CTGCTTATC |          |
|       | GGCATTTAG | GAAGTATTA | TGCACTTAT | GGCATTTAG | GGCATTTAG | GGCATTTAG | GGCATTTAG | GGCATTTAG | GGCATTTAG | GGCATTTAG |          |
| 26301 | TAGCAAGAT | CTGCAAGAT | CTGCAAGAT | CTGCAAGAT | CTGCAAGAT | CTGCAAGAT | CTGCAAGAT | CTGCAAGAT | CTGCAAGAT | CTGCAAGAT |          |
|       | ATCTCTCTA | GAATCTCTA | GAATCTCTA | GAATCTCTA | GAATCTCTA | GAATCTCTA | GAATCTCTA | GAATCTCTA | GAATCTCTA | GAATCTCTA |          |
| 26401 | CTTAAAGAA | GGATTTTTC | GGATTTTTC | GGATTTTTC | GGATTTTTC | GGATTTTTC | GGATTTTTC | GGATTTTTC | GGATTTTTC | GGATTTTTC |          |
|       | GAATCTTTT | CTTAAAGAA | CTTAAAGAA | CTTAAAGAA | CTTAAAGAA | CTTAAAGAA | CTTAAAGAA | CTTAAAGAA | CTTAAAGAA | CTTAAAGAA |          |
| 26501 | CCCGGCTTC | CTTAAAGAA | CTTAAAGAA | CTTAAAGAA | CTTAAAGAA | CTTAAAGAA | CTTAAAGAA | CTTAAAGAA | CTTAAAGAA | CTTAAAGAA |          |
|       | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC |          |
| 26601 | CTAGTTTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC |          |
|       | GATCAAGAG | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC |          |
| 26701 | GAATTTTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC |          |
|       | CTTAAAGAA | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC |          |
|       |           |           |           |           |           |           |           |           |           |           |          |
| 26801 | GAATTTTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC |          |
|       | CTTAAAGAA | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC |          |
| 26901 | TCGCGGCTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC |          |
|       | AGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC |          |
| 27001 | TCAGGCGGC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC |          |
|       | AGTCCCGCG | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC |          |
| 27101 | ACGAGTCTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC |          |
|       | TTCTTCTCA | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC |          |
|       |           |           |           |           |           |           |           |           |           |           |          |
| 27201 | TCGCGAGAT | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC |          |
|       | AGAGTCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC | GGCGGCTTC |          |

Figure 150

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|       |                                                                                                                                  |
|-------|----------------------------------------------------------------------------------------------------------------------------------|
| 27301 | CTCTCCGGCC ACTATCCGGA TCAGTTATTT CTATACCTTG AGCTGTATTA GAGCTCTGCG GACGCTCTAG ACTGAATGTT AAGTGGGAGG GCAGAGCAGC                    |
| 27401 | GGAGGGCCGG TGATAGCCCT AGTTAAATTA GATATGAAC TCTCTATTTT CTTGAGCTCT CTTGCTATTC CTGCTATTCG ATATCTGAGT                                |
| 27501 | TGCGCTGAA ACACCTGGTC CACTGCTGC GGCACATGTC CTCTGCTCTT GACTTCGGTG AGTTTTCCTA CTTTGAATTC CCGAGAGTAC                                 |
|       | ACCGGACTT TGTGACCCAG GTGACATCG CGGTGCTTAC GAAATGCTCG CTTGAGGCCAC TCMAACGAT GAACTCTTAC GGGCTCTTGG TATGCTCTCT                      |
|       | CCCGGCGAC GGCCTGCGG TTACCGGCA GCGAGAGCTT GCTGCTATCT TGAATTCGGA GTTATACCGG CCGCCCTCTG TAGTTGAGCG GGCAGGCGTA                       |
|       | GGCCGCGG GCGCAGCGCG AATGCGGGT CCTCTCGAA CGCGATCGG ACTATGCTCT CAAATGGGTC GCGGGGAGG ATCAACTCGC CCGTCCCTCT                          |
| 27601 | CCCTGTGTC TCACCTGAT TTGCACCTG CCTAACCTG GATTACATCA ACATCTCTCT TCCCATCTCT GTGCTGATTA TAATAAATAC AGAATTAATA                        |
|       | GGACACAG AGTCACACTA AACGTCTACA GGAATGGAC CTATATATCT TCTAGAAACA ACGGTATAGA CAGACTCAT ATTATTTATG TCTTTTAT                          |
| 27701 | ATATACCTGG GCTCTCTATG CCATCTCTGA AAGCTCACCG TCTTACCGG CCTACCGG CCAATGCGAA CCAATGCGAA CCAATGCGAA ATCTCTCC                         |
| 27801 | TATATGACCC CGAGATAGC GGTATGACAT TTGCGTCTGC CTACCTCAGA TGTCTCTTGG GAGAGCTCG AGCTGATGAG CTATGCTCTT TGTGCTGCG ACGATATGAG            |
| 27901 | CTGTGATTTA CACAGTTTC AACCCAGAG CAGTGTGCTT ACTATGAGAC ACTTCCGAGC TCTGCTGAG CTATGCTCTT TGTGCTGCG ACGATATGAG                        |
|       | GTACTAAAT GTTGTCAGAG TTGCGTCTGC CTACCTCAGA TGTCTCTTGG GAGAGCTCG AGCTGATGAG CTATGCTCTT TGTGCTGCG ACGATATGAG                       |
| 28001 | CGCGATGCTT ACGATGCTCT CACCGCGCG TTGCGTCTGC CTACCTCAGA TGTCTCTTGG GAGAGCTCG AGCTGATGAG CTATGCTCTT TGTGCTGCG ACGATATGAG            |
|       | GGCCCTTCCA TGTCTACGCA GTGCGCGCG AGCTGCTGAG TTGCGTCTGC CTACCTCAGA TGTCTCTTGG GAGAGCTCG AGCTGATGAG CTATGCTCTT TGTGCTGCG ACGATATGAG |
|       | AACAGAGCTT GAGCTTACGA AACCTTACG GTATATGAGG AATGCTCTAG CTACTGCTG GTTATGAGC AATTCAGGCA ACTCTAGCGG CTATCTCTAT                       |
|       | TGTCTCTCCA CTGCGATCTT TTGGGAATCC CATATCCCG TTTCGCGCTC GATACACCC CAATATCTTG TTAATGCTCT TGAATATGCC GATATGAT                        |
| 28101 | TCAGCTTTCT CTAGATAGCG GCTTGGGTT ATTCTCTG ATCTGATCTT CTATATCTT ATACTAAGC ATACTAAGC TCTCTGCTT AAGCTGCGC GCTCTCTT                   |
|       | AGTCCAAAGA GATCTTAGCC DCAAGCCCAA TAAGAGACAG AACACTTANG GAATATGAA TATGATTCG ATACTAAGC TCTCTGCTT AAGCTGCGC GCTCTCTT                |
| 28201 | TGCACATTTG CATTTATTTT CAGCTTTTTA AAGCTTGGG TCGCCACCCA AGATGATTAG GTACATATC CTAGCTTAC TCAGCTTAC GCTCTCTT                          |
|       | ACGTGTAAAC GTATATACCA GTCGAANAAT TTGCGACCCC AGCGTGGGT TCTACTAATC CATGTATTAG GATCCAAATG AGTGGGAGCG CAGTCTGGGT                     |
| 28301 | GATACCAAGC AATGCTGGA TTTTAAGGAG CAGCTCTGTA ATGTTACAT CTGAGCTGAA CCGAGCTGAA GCTATGCTT GCTCTGACTT GCTCTGCTT                        |
|       | CCATGGTGGG TTTTCCACCT AAAATCTCTC GGTGCGCAT TACATCTTAA GCTCTGACTT GCTCTGACTT GCTCTGACTT GCTCTGACTT GCTCTGACTT                     |
| 28401 | ATGAAAGCT GCTTATTCG CACAAACA AAATTCGCA GTATCTGTT TATGCTATTT GCGAGCCAGG TCACACTACA CAGTATATG GAGTATATG TTACAGTTT                  |
|       | TACTTTTCCA CGAATAAGCG GTGTTTTTGT TTTTACCGTT CATACGACAA ATACGATPAA CCGTGGTCC ACTGTGATGT CTCATATTAC AATGTCAGAA                     |
| 28501 | CCAGGCTTAA AGTCATPAA CTTTATGTA TACTTTTCCA TTTTATGAA TGTGCGCAT TACCATCTAC ATCAGCAAAC AGTATATGTT GTGCGCCCCA                        |
|       | GGTCCCATTT TCAATATTTT GAAATACAT ATCAAAAGGT AAATATCTT ACACGCTGTA ATGCTATCTG TACTGCTTGT TCAATATCAA CAGCTGCTT                       |
| 28601 | CAAAATTTTG TGGAAACAC TGGCACTTTC TGTGCACTG CTATGCTTAT TACAGTCTC GCTTGTGCT GTACCTTACT GTACCTTACT GTACCTTACT                        |
|       | GTATTAACAC ACCTTTTGTG ACGGTAAAG ACGAGTGAC GATACGATTA ATGTCACAG CGAAACGAGA CATGGATGA GATATATTT ATCTTTCT                           |
| 28701 | GACGAGCTT TATTTAGGAA AAGAAATGC CTTAATTTAC TTAGTTACAA AGCTAATGTC ACCACTACT GCTTACTGCG CAGCTTCTAA AICAAATTT                        |
|       | CTGGTCTGAA ATAACTCTT TTCTTTTACG GAATTAATG AITCAATGTT TCGATTTACG TGTGATGA CGAATATGCG GAGTATGCTT TGTGTTAT                          |
| 28801 | AAAGTTAGC ATTATATTA GAATAGATTT TAAACCCCC GGTCAATTC GGTCAATTC TCTCAATTC TCTCAATTC AICAAATTT                                       |
|       | TTTTCATATG TAATATTAAT CTTATCTTAA ATTTGCGGGG CCAATTAAGG ACGAGTTATG GTATGCGGAC TTGTTACTG AGATACACCC TATACGAGT                      |

Figure 15R

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|       |                                                                                                                                                                                                                          |
|-------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 28901 | GGCTACAC CTTGAGTCA GCTTCTCTG ATCTAGCAT CTGACTTGG CCAGGACCTG TCCCGCGCAT TGTCTCAGT CCACTACAG CCAACCCACTC<br>CCGATGTTG GAATTCAGT CCGATGATC TACATGATG GACTGAAAC GGTGTGTATC AGGCGCTCA ACGAGCTCA GCTTATGTC GCTTGTGAT           |
| 29001 | TAAACAGCAT GACCAACACA ACCACGCTG CCGCGCTTAC CCGACTTACA TTACACACA ATACACCCCA AGTTCTGCC TTCTCTGATA ACTGAGATA<br>ATTCTCTCA CTGCTTCTGT TGTCTGCTG GCGCGCTG GCTGATCT GCTGATCT AGATGCTT TATGTGCTT TCAAGACCG AACAGCTTAT TCACTCTAT |
| 29101 | CTTGGCAGC TGTGCTTCT CCATAGCTT TATGTTTGA TCACTTATTA TTATGCTCT CATCTGCTG CTAAAGCCCA TTGCGCGGC TCGTCTATC<br>GAACCGTAC ACCACCAAGA GGTATGCGCA ATACCAACAT ACCGATAT TACACAGCT GTAGACAGG GATTTGCGT TCGCGCGGC TCGTCTATC           |
| 29201 | TATAGTCCA TCAATGCTT ACACCCAAAC ATATATAT TATATAGAT GATCGCAT GTAGACAGG GATTTGCGT TACAGTATCA TTAAATAGCA<br>ATATCAGCT AGTACACCA TGTGCTTGG TTTACTACTT AGCTATCTTA CCGCGCTG ATCTGTACA AGAAAGAGA ATGTCTACT AATTTACTT             |
| 29301 | CATGATCTT CAGCTTTTA TATTACTGAC CTTGCTGCG CTTTTTGG GGTCTCTAC ATTGCTGCG GTTCTTACA TCGATGCA CTGATTC A<br>GTACTAAGCA GCTCAAAAT ATATGACTG GGAACACCG GAAACACAC GACCGATG TACCGACCG CAAGAGTGT AGCTTCACT AGCTTACG-T               |
| 29401 | GGCTTACAG TCTATTGCT TTAGGATTT GTACCCCTCA CCGTATCTG CAGCTTATC ACTGTGCTA TCGCTTAT TCGCTGAT GACTGCTT<br>CGAGATGTC AGATTAAGCA ATGCTTAA CAGTGGAGT GCGATGAGC GTGATGAG TACACAGT AGCCAGAT ACCGAAATA GGTACAGTAA CTGACCCCA         |
| 29501 | GTGCGCTT TGCATATCT AGACCCATC CCGATGAC GAGCGACT ATAGTACG TCTTATGAT TCTTATTA TGAATTTAC TGTGCTT<br>CACACCGAA ACCATATAG TCTGTGCTAG GGTCTATCT CCGTCTCTA TATGACTCG AGATATCTA AGAATTTAT ACTTTAAATG ACATGAA-A                    |
| 29601 | CTGCTGATTA TTGCACTT ATCTGCTTT TGTGCTGCA CCGTCAAGC TCAAGACAT ATATCAGCA GATTCATCG TATATGAT ATTCAGCT<br>GACGACTAT AACGTGCGA TACACCGAA ACAGCGCTT GAGCTTGG AGTTCTGTA TATAGTCT CTAAAGTAC ATATACCTTA TAAAGTCTCA                 |
| 29701 | GCTACATGA AAGAGCAT CTTTCCGAG CCGCTTATA TCGATATC TCTGTATG TGTCTGCG TACCATCTA GCGCTAGCTA TATATGCC-A<br>CGATGTTACT TTTTCTCTA GAAGGCTTC GATCAATAT AGCTTAGTAC AGACAGCT ACATGAT ATGATGAT CCGATGAT ATATAGCAT                    |
| 29801 | CCTTGACAT GGTGGAAG CAATGATG CATGACAC CCGCTTTTC CCGCGCTG GCGCGCTG ATACAGCT GAGTCTCTT AACACGCGC GCGTCTG<br>GAACTGTA CCGACTTGC GTTATCTAG GTACTGCTG GTTCAAGG GCGCGCTG ATACAGCT GAGTCTCTT AACACGCGC GCGTCTG                   |
| 29901 | CCAGCCATC AGCTGCTC ACCCTGCTC TCGAGAGCG TCGGCTGAC TTACTGCTT GAAATTTAGT TGTCTCTCT TACTGCTT GCGATCTA TCTTTTACT<br>GGAATTTAT CAGAGAGCG CCGCTAGAA AGACGACG CAGCGCTG GATGATGAT ATGATATCAG ACTTCAAG CATGCTTAC TTGACCATG         |
| 30001 | CTTAAATAT GTCTGCTG GAGCATCT TCTGCTCC GTCGCTGCT GTCGCTGCT TACTTATTC TCGAGCTCT GTACCAATG AACCTGCTA<br>GCAAGCGG TATCTTTGT CTGTAAGC AGTCAAGT CACCTAGCA AGTATACCA CCGACACCG CCGTACTAC AGTTGCTCA CCAAGCTT-A                    |
| 30101 | CGTTTCCC ATAGAAACA GAGCATTTG TCGGCTTCA GTGATGCT TCAATATCT GCGCTGCT GCGATGCTT TCGACAGT GGTCTTAT<br>GAAATGCT GTCATGCTG CAGAAAGC CATTAACATA ACTGACCT TATATATC CCGAGCTG CCGAGCTG ATTCATGAC CTGTCAAGS ACCTGATG                |
| 30201 | CTTTAACCA CAGTACCAC CCGTTTCTG GTATGCTAT TCGATGCTA GCATCTTTC GCTTCCAG TAACTGCTG GAACATGCT TCGACTCTA<br>CTCTGACCC TTATTAGAC CCGTGTGCT TATGCTCT TAACTATTA AAAAAATA TAAAGCTCA CTTACTTAA ATCAGTTAGC                           |
| 30301 | GAGAGTGG ATATATCTG GACACGCA CAGTTCTTAT AATAGGTA ATGATAT TTTTTTAT ATTCTGCT GATGATAT TACTCAATG                                                                                                                             |

Figure 155

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30401 AATTTCTGT CCAGTTTAT CCAGACACC TCTTTGCTT CCTCTCAGCT CTCTCTATTG AGCTTCTCTC TGGCTGCAAA CTTCCTCCAC ATCTTAATG  
 TTTAAAGACA GGTCAATATG GTCTCTGTG AGCAACCGGA GAGAGCTTGA CAGCTATAGG TCGATAGAGG ACCGACCTTT GAAAGAGCTG TTACATTTAC  
 30501 GAATGTGAGT TTCTCTCTGT TCTCTCTCAT CCGTACCCAC TATCTTTGAG TATCTTTGAG TGAAGCGGC AAGTACCTCT GATCATCTCT TCAATCCCTCT  
 CTTACAGTCA ACGACAGACA AGCAAGAGTA GAGCTGTGAG ATAGACATG ACNACCTCT ACTTCTGCG TCTCTGAGCA CTCTCTATGA AGTTGCGG A  
 30601 GTATCCATAT GACACCGAAA CCGTCTCTCC AACTGTCTCT TTCTTTACTT CTCTCTTTGT ATCTCCCAAT GGGTTTCAAG AGATCTCCCTC TGGGTACTI :  
 CATAGGTATA CTGTGCTTTT GGCACGAGG TTGCACAGGA AAGATATGAG GAGCGAACA TGGGGGTTA CCAAAATTC TCTCAGCGGG ACCCCATGAG  
 30701 TCTTTGCGCC TATCGAAC TCTAGTTTACC TCCATGCGA TGTCTGCTCT CAAATATGAC AACTGTCTCT CTTCTGAGCA GGCCTGAGC CTTACCTCTCT  
 AGAAACCGGG ATAGGCTTTGG AGATCAATGG AGCTTACCTT ATCTAACCTA GTTTTACCGG TTCCCGGAGA GAGACCTCTCT CCGGCTCTTG GAATGAGI :  
 30801 AATATGTAC CACTGTGAGC CCACCTCTCA AAAAAACCA GTTAAATATA AACCTGTAAA TATCTGTACC CCTCACAGCT ACCTACAGAG CCTTAACCTCT  
 TTTTACATG GTACACCTGG GTTGTGAGT TTTTGTGCT CAGTTGTAT TTGACCTTTT ATAGAGCTG GAGTGTCTCA TGGAGTCTTC GGGATGTGACA  
 30901 GGTCTGCGCC GCACCTCTTA TGGTCTGCGG CAACACATC ACATGCAAT CACAGCGCC GCTAACCTG CACGACTCCA AACTTAGCA TGGCACCCTA  
 CCGACCGCGG CGTGTGAGT ACCAGCGCCC GTTGTGTGAG TGTGTGCTTA GTGTCCGGG CCAATGCGAC GTGCTGAGCT TTGAATCTTA AGGTGCGI :  
 31001 GAGCCCTCA CAGTGTGAGA AGGAACCTA GGCCTGCAAA CATTACGCCC GTCTACCTTAC TATCAGTCC TATCAGTCC TATCAGTCC TATCAGTCC  
 CTTGTGAGT CACTGTGAGC TTGGCATGG ACTTGAAAGA GCGCATTTAT ACACAAATG GAAACTAGG ACTTAAGTAC GGGGTCTCTT TGCATGTAT  
 31101 ATGTATGAG GTGACCATG AACCGTATC TGAATCTTCT CAGGTAAATA TGTCTTTTAC CTTTGTGAG TGAATCTTAC CCGCGAGGA AGTATCATTT  
 31201 AGAGACCTA AACTTTTGA CCGTAGCAAC TGGTCCAGT GTCACTATTA GTCACTATTA ATAACTCTT CTTGCAACT GAGCTTGGG TTTTGTATTTA  
 TCTCTGAT TTGTGAACT GGCATCTTG ACAGGTCCA CACTGTAA TATTATGAG GAGCTTTGA TTTCAATGAC CTGCAACCC AACTATAT  
 31301 CAAGGCAATA TGCACCTTAA TGTAGCAGGA GCACTAAGGA TTAATCTCA AACTAGTCA AACTAGTCA TACTATGAG TACTATGAG TACTATGAG  
 GTTCCCTTAT AGGTGTAAAT ACATCTCTCT CTTGATTTCT ACTTAGAGT TTGTCTCGG GAATATGAG TACTATGAG TACTATGAG TACTATGAG  
 31401 AACTAAATCT AGACTAGGA CAGGCTCTCT TTTTATATA CTAGCCGAC AACTGTGATA TTAATGAGA CAAGGCGCTT TACTTGTATA CAGCTTCAAA  
 TTCAATTTAGA TCTGTATCTT GTCCCGGAG AAAAAATTTT GAGTGGGTG TTGAACCTAT AATTGATCTT GTTTCCGGA ATGAACAAAT GTCCAAATTT  
 31501 CAATTCAAA AGCTTTGAGG TTAACCTTAG CACTGCGAAG GGTGTGATGT TTCAACCTAC AGCCTAGCC ATTAAATGAG GAGATGGCT TGAATTTCTT  
 GTTAGGTTT TTCAACTCT AATTGCTATC GTAGCGCTTC CCAACTACA ACTGTGATG TCGTATCTG TAATTACTG CTCTACCGA ACTTAACCA  
 31601 TCAGCTAATG CACCAACAC AATCTCCCTC ABAACAAA TTGTGATG CCTAGAAATTT GATTAACCA AGCTATGCT TCTTAACCTA GGAATCTCT  
 AGTGTATTAC GTGTTTTTGT TTTAGCGGAG TTTTGTGTTT AICGCTAGC GGAATCTTAAI CTAAATTTGT TCCATATCA AGGATTTGAT CTTTACCTG  
 31701 TTAGTTTTGA CAGCAGAGT GGCATTTAG CCGTATGCT ATCTTTGCT TTTATTTACT TTTGATGAGT TGTGAGCTT TGTGAGCTT TGTGAGCTT  
 AATCAAACT GTCTGTCCA CCGTATGCT CCGTATGCT ATCTTTGCT TTTATTTACT TTTGATGAGT TGTGAGCTT TGTGAGCTT TGTGAGCTT  
 31801 TGCAGAGAA GATCTAAG TCACTTTGCT CTTAACAAA TGTGCTACT TTAATTTACT TTAATTTACT TTAATTTACT TTAATTTACT TTAATTTACT  
 ACCTCTCTT CTAGATTTG AGTGAACCA GATTTGTTT ACAGCTGAG TTTATGAGG TTTATGAGG TTTATGAGG TTTATGAGG TTTATGAGG  
 31901 ATATCTGAA CAGTTCAAG TGTCTCTCT ATTAATGAT TTGCGAATA TGTAGTCTA CTAAACAAAT CTTCTCTGGA CCAAAATAT TAACTTTA  
 TATAGACTT GTCAAGTTTC ACGATGAG TAAATTTCTA AACTGCTTTT ACCTACAGT GATTGTGTA GGAAGGAGCT GGTCTTTATA ACCTTCAAT  
 32001 GAATGTGAGA TCTTACTGAA GGCACAGCT ATCAACGC TGTGTGATTT ATGCTTACG TATCAGCTTA TCCAAATCT CAGGTAAAA CTGCCAAGG  
 CTTTACCTCT AGATGACTT CCGTGTGGA TGTGTTGCG ACACCTAATA TACGATGAG ATAGTCAAT AGTTTATAGA GTGCCATTTT GACCTTTTTC

Figure 15T

DHKAA5999 MER512

32101 TAACATGTC AGTCAGTGT ACTTAAAGG ACAGAAAGT AAACCTGTGA CACTAACAT TACACTAAC GGTACACAG ANACAGNGA CACAACCTA  
 ATTGTACAG TCAGTTCNA TGAATTTGG TCTGTTTTA TTGTAACAT GTGATTTGA ATGTAATTT CCAATGTCC TTGTCTCTT TTTGTTAGT  
 32201 AGTGCATCT CTATGTAT TCCATGGAC TCTCTGCTT ACATATCAT TAATGAATA TTGTCACAT CTTCTACAC TTTTTCATAC ATTGCCCAN  
 TCACGTATCA GATACAGTAA AAGTACCTG ACACAGCCG TGTTCATATA ATTACTTAT AACGTTGA GGAATATG AUAAGTATG TACGXTT  
 32301 AATAAGAAAT GGTGTGTG ATGTTTCAC GTTTTATTT TTAATTTTA GAATTTTGA ATCATTTTTT CATTCAGTAG TATAGCCCA CCACACATA  
 TTAATTTCTA GCAAACACA TACAAAGTTC CACAAATNA AAGTAAAGT CTTTAAAGT TCAGTAANA GTAGATCAT ATATCGGCT GTTGTGT  
 32401 GCTTATACAG ATCAGGTAG CTTAATCNA CTCACAGAC CTAATGATTC AATCTCAC CTCCTCCCA ACACACAG TACACAGTCC TTTTTCCT  
 CGAATATGTC TAGTGCATG GAATTAATTT GAGTGTCTT GATCATAG TTGATGCTG TTAGAGCTG TGTGTGCTC ATGTGTGAG ANAGGG  
 32501 GCTGGCTTAA AUAAGATCA TATCATGGT AACACATA TTCTTATG TTATTTCCA CAGGTTTTT TGTGAGGCA AACGTCATC AGTGTATTT  
 CGACGGAAT TTTTGTAGT ATAGTACCA TTCTGTAT ACATATCCAC AATATAAGT GTGCCAAGG ACAGTCTGT TTGCGAGTAG TCATATAT  
 32601 ATAAGCTCC CGGCAGTTC ACTTAAGTC ATGTGCTGT CTATCTGTC ARCCAGGC TGTCTGCA TTTGCTGTG CTTAACGGC GGTACAGTA  
 TATTTGAGG GCGGTGAG TGAATTCAG TACAGCA CAATCTGAC TGTGTGCTG ACACAGGTT GAAGCCGAC GAATTCGCG CCGCTTCTT  
 32701 AAGTCCAGC CTACATGAG GTAGGTCT ATGTGTCAT CAGATAGG GTGTGTCT GCAGCAGGC GGGATTAAC TGTGTGCGC GCGGTCTCT  
 TTCAGGTGG GATGTACCC CATCTCAGT TTAGCAGTA GTCTATCCC GCACACCA CGTGTGCTG GGTATTTTG ACAGAGGCG GCGCGAGCA  
 32801 CCTGAGGAA TACAAATG CAGTGTCT CTCAGCAT ATTCGACCG CCGGAGCAT AAGGCTCTT GTCTCTCGG CACAGCAGG CAGCTGTAT  
 GAGCTGCTT ATGTTGTACC GTACACAG GAGTGTCT TAAGGTTTC GGGGTGCTA TTCCGCGCA CAGGAGGCC GTGTGCTGC GTGAGCTT  
 32901 TCACCTAAAT CAGCAGCTA ACTGAGCAC AGCAGCACA TATTTTCTA ATCTCCAG TGCAGGCG TGTATCCAA GTTCATGCG GGGACACAG  
 AGTGAATTTA GTCTGTCT GTAGTCTGT TGTGTGTT ATACAAAGT TTAGGCTTC AGTTTCCCG ACATAGTTT CGATACCGC CCGTGTGTC  
 33001 AACCCAGTG GCTATCAT CACAGCGCA GTTAGATTA GTTGTATCC CTCATNACA GCTGTGACAT AACATTAAC TCTTTTCCA TGTTCATAA  
 TTGATGACAC CGTATATG GTGTGCGT CATCTAAT CAGGCTGAG GAGTATTT GTGACCTGTA TTTGTAAATG AGAAACCGT ACATCATTA  
 33101 CAGCAGCTCC GGTATCCATA TAACTCTG ATTAACATG GCGCATCCA CAGCATCTT AACCATGCT GCGAAACCT CCGCCTCGC TATACACTA  
 GTGATGAGG GCGATGCTAT ATTGAGAC TAATTTCTAC CCGGTAGT GTGTAGGA TTGTGTGAC CCGTGTGCA CCGCGCGCG ATATGTGAC  
 33201 AGCGAACCG GACTGGACA ATGACAGT AGAGCCAG ACTGTAAAC ATGATCATC ATGCTGTGA TGTATCAAT TGTGGCACA CACAGCACA  
 TCCCTTGCC CTGACCTGT TACTGTCC TCTCGGTC TGACATG TACCTAGTAG TACGAGCAT ACTATAGTTA CAGCGTGT GTGTGCTGT  
 33301 CTTGCTACA CTTGCTCAG ATTACACT CTTCCGCTT TACAGCATA TCCAGGGA CAGCCATTC CTGAATCAG GTAAATCCA CACTTCAGG  
 GACGTATGT GAGGAGTCC TAATGTTGA GAGGCGCA ATCTGTAT ATGTCTCTT CTGTGTGAG GACTTGTG CATTTAGGTT GTGACCTC  
 33401 AGACCTGCG AGTAACCTA CTTGTGCT TGTCAAGCA TTAGATTCG GAGCAGCT ATGATCTCC AGTATGAG CCGCGTTTC TGTCTCMA  
 TTCTGGAGG TCCATTCAGT GCACAGCTA ACATTTTAC ATGTAAAGC CTTCTGTC TACTAGTAG TCATACCAT CCGCCCAAG ACAGAGTTT  
 33501 GAGGTAGAC GATCCCTACT GTACGATG CCGTATACA ACCGAGATG TTTTGTCT ATGTCTATC CAATGTGAC CCGGACCTA GTCTATTT  
 CCTCCATCT GTAGGATGA CATCCCTAC CCGCTCTGT TGGCTTAGC ACATACCA TCACAGTAG GTTTACCTG CCGCTGCT CATGTATAA

Figure 15U

## pMRKId5gag MFR682

|       |                                                                                                               |
|-------|---------------------------------------------------------------------------------------------------------------|
| 33601 | CTGAGACAAA ACCAGGTGCG GCGCTGACAA ACAGATCTTC GTCTCTGCTC TTTCTGCTTC TATAGTAGTT GTATATATAT CACTCTCTTA            |
| 33701 | GACTTCCTTT TGGTCCAGGC CCGCATCTTT TGTCTAGACG CAGAGTCCAG AGCGCGAT CTATGATATG CATCATATAT GTAGAGATTT              |
| 33801 | AAGCATCCAG GCGGCGCTTC GCTTCGATTT CTATGTATTC TCTCTATGTC GCGCTCTCCC TATATATATC CATTATATAT CACCCAGCTC            |
| 33901 | TTCTATAGTC CCGCGCGCAC CGAAGCCCAA GATACATTTG AGTAATATAG CCGCGAGCGG GATATATATC TATATATATC AATCTTATAT            |
| 34001 | ACCTACACAT TCGTCTCTCG AGTCACACAC GCGAGTATCG GGAATATATC GATACACAT TATATATATC TATATATATC AATCTTATAT             |
| 34101 | TGGATGTGTA AGCAAGAGCC TCAGTCTGTC CCGTCTCTCG CCGTCTCTCG CCGTCTCTCG CCGTCTCTCG CCGTCTCTCG CCGTCTCTCG            |
| 34201 | ATGAGATCTT ATTAAGTCAA CCGCTCTCCC TCGCTCTCG TCGTCTCTCG TCGTCTCTCG TCGTCTCTCG TCGTCTCTCG TCGTCTCTCG             |
| 34301 | TACTTCTAGA TAATTCATTT GCGCGAGCGG AGCGGAGCGG AGCGGAGCGG AGCGGAGCGG AGCGGAGCGG AGCGGAGCGG AGCGGAGCGG AGCGGAGCGG |
| 34401 | TGCAAAAGGC AAGCGGCTTT CAGCTCCAG TCGAGGTAAA GGTATATATC TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC             |
| 34501 | AGGTTTTCCG TTGCGCGGTA GTGCGAGTTC ACTGCGATTT CCGATTTCCG AAGTCTCTCT TATATATATC TATATATATC TATATATATC            |
| 34601 | ATAAATCTTC ATCTGCGCAC GTTCTCTATA TATCTTAAAG CAAATCTCTG TATATATATC TATATATATC TATATATATC TATATATATC            |
| 34701 | TTATTTAAGAG TAGAGCGGTC GAAGGTATAT ATAGAGTTC TATATATATC TATATATATC TATATATATC TATATATATC TATATATATC            |
| 34801 | CAGGCTCAG CAGCGAATCA TGAATTCGAA AATTCAGTTT CCGTCTCTCG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC             |
| 34901 | GTGCGAGTTC GTGCGAGTTC TGAATTCGAA AATTCAGTTT CCGTCTCTCG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC            |
| 35001 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 35101 | CGAAGGAGGC GTGCGGAGTC TGAATTCGAA AATTCAGTTT CCGTCTCTCG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC            |
| 35201 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 35301 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 35401 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 35501 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 35601 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 35701 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 35801 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 35901 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 36001 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 36101 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 36201 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 36301 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 36401 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 36501 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 36601 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 36701 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 36801 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 36901 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 37001 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 37101 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 37201 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 37301 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 37401 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 37501 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 37601 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 37701 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 37801 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 37901 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 38001 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 38101 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 38201 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 38301 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 38401 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 38501 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 38601 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 38701 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 38801 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 38901 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 39001 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 39101 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 39201 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 39301 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 39401 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 39501 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 39601 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 39701 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 39801 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 39901 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |
| 40001 | CGTCTCTCTG CAGCGGAGCG TGAACATAT GTTCTCTCTG TCGAGGTGTA ATCTCTCTTA TACATATATC AGCATATATC AGCATATATC             |

Figure 15V

[illegible]

40/144

pMRKd5gag MER682

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37001 CACACCGGA TAAATCCGG CCACATAGCA GAACTTTAA AGTCTCATC ATTGAAAC GTCTTCGG GCGAAACTC TCAGGATCT TACTGCTTT
GTGTGCGCT ATTATCGGC CACTCGTCC GGTGTATGTT CTTCGAAATTT TACGACTAG TACCTTTTG CAGAACCC CCGTTTGAG AGTTCCTAGA ATGCGAGAA
37101 GAGATCCAGT TCGATTTAAC CCACTCGTCC GGTGAGCAGT TCGTTCAGT AGAAGTCTA GAATTCGAA GTCTCTGCA GTCTCTGCA GAGGCAAAAT
CTCTAGGTCA AGCTACATTC GGTGAGCAGT TCGTTCAGT TCGTTCAGT TCGTTCAGT TCGTTCAGT TCGTTCAGT TCGTTCAGT TCGTTCAGT TCGTTCAGT
37201 GCGGCAAAA AGGGAATGAG GCGGCAAGG AATGTTTGA TATTCATCT ATTACATTC GAAAGAAA GTTATATTA GTTATATTA GTTATATTA GTTATATTA
CGCGGTTTTT TCGCTTATTC CCGCTGCGC TTTACATCT ATTACATTC AGGCTTTC CCGCAATTC CCGCAATTC CCGCAATTC CCGCAATTC CCGCAATTC
37301 GCGGATACAT ATTGAATGT ATTAGAAA ATAAACAAAT AGGCTTTC CCGCAATTC CCGCAATTC CCGCAATTC CCGCAATTC CCGCAATTC
CGCTATGTA TAACTTACA TAACTTCTTT TATTGTTA TCCCAAGGC GCGTGAAG GCGCTTTC CCGTGGACT CAGATCTTT GTTATATTA

```

BamHI  
 EcoRI

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37401 CATGACATTA ACCTATAAA ATAGCGTAT CAGTACGCC TTCTGCTTC AGCAATGGA TCGAATCT TAAT (SEQ ID NO: 27)
GTACTATAAT TGGATATTTT TATCCGATA GTCTCGCG AAGCAGAG TTCTTACCT AGCCTAAGA ATTA (SEQ ID NO: 28)

```

Figure 15X



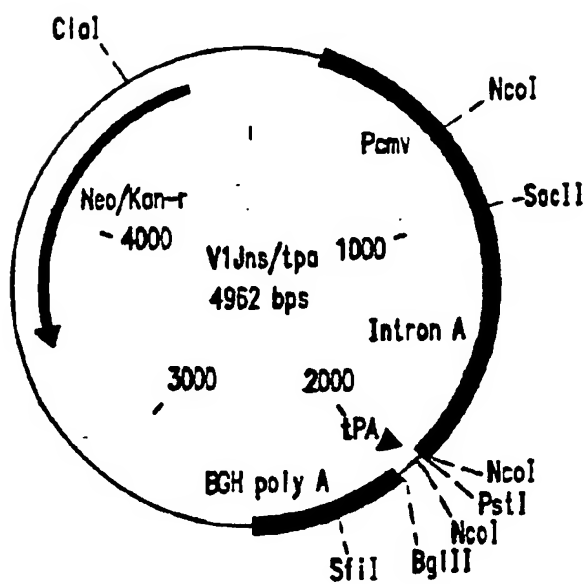
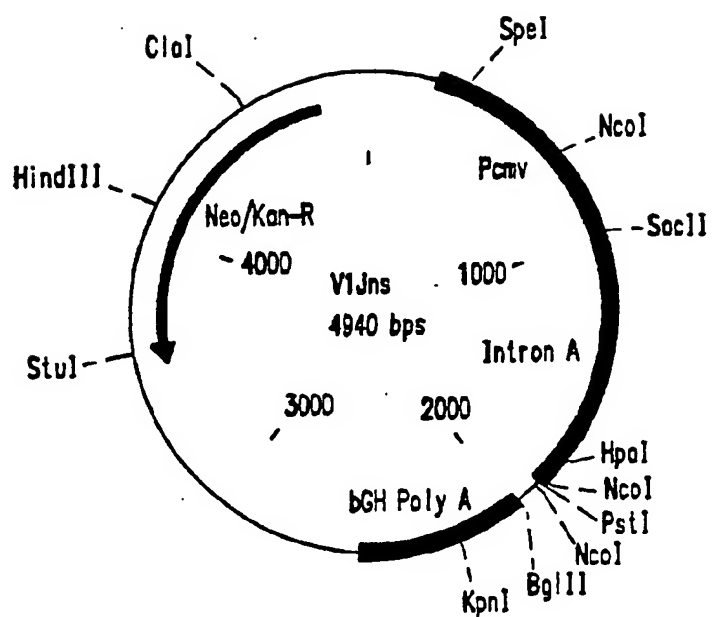


FIGURE 16

AGATCTACCATGGCCCCCATCTCCCCATTGAGACTGTCCCTGTGAAGCTGAAGCCTGGCATGGATGGCCCCAAGGTGAA  
 Bg/II MetAlaProIleSerProIleGluThrValProValLysLeuLysProGlyMetAspGlyProLysValLys  
 1 10 20  
 GCAGTGGCCCTGACTGAGGAGAAGATCAAGGCCCTGGTGGAAATCTGCACTGAGATGGAGAAGGAGGCAAAATCTCCA  
 sGlnTrpProLeuThrGluGluLysIleLysAlaLeuValGluIleCysThrGluMetGluLysGluGlyLysIleSerL  
 30 40 50  
 AGATTGGCCCGAGAACCCTACAACACCCTGTGTTGCCATCAAGAAGAAGGACTCCACCAAGTGGAGGAAGCTGGTG  
 ysIleGlyProGluAsnProTyrAsnThrProValPheAlaIleLysLysLysAspSerThrLysTrpArgLysLeuVal  
 60 70  
 GACTTCAGGGAGCTGAACAACAGGACCCAGGACTTCTGGGAGGTGCAGCTGGGCATCCCCACCCCGCTGGCCTGAAGAA  
 AspPheArgGluLeuAsnLysArgThrGlnAspPheTrpGluValGlnLeuGlyIleProHisProAlaGlyLeuLysLys  
 80 90 100  
 GAAGAAGTCTGTGACTGTGCTGGCTGTGGGGATGCCTACTTCTGTGCCCCCTGGATGAGGACTTCAGGAAGTACACTG  
 sLysLysSerValThrValLeuAlaValGlyAspAlaTyrPheSerValProLeuAspGluAspPheArgLysTyrThrA  
 110 120 130  
 CCTCACCATCCCTCCATCAACAATGAGACCCCTGGCATCAGGTACCACTACAATGTGCTGCCCCAGGGCTGGAAGGGC  
 loPheThrIleProSerIleAsnAsnGluThrProGlyIleArgTyrGlnTyrAsnValLeuProGlnGlyTrpLysGly  
 140 150  
 TCCCCTGCCATCTTCCAGTCTCCATGACCAAGATCCTGGAGCCCTTCAGGAAGCAGAACCCCTGACATTGTGATCTACCA  
 SerProAlaIlePheGlnSerSerMetThrLysIleLeuGluProPheArgLysGlnAsnProAspIleValIleTyrG  
 160 170 180  
 GTACATGGCTGCCCTGTATGTGGCTCTGACCTGGAGATTGGGCAGCACAGGACCAAGATTGAGGAGCTGAGGCAGCACC  
 nTyrMetAlaAlaLeuTyrValGlySerAspLeuGluIleGlyGlnHisArgThrLysIleGluGluLeuArgGlnHisL  
 190 200 210  
 TGCTGAGGTGGGGCTGACCAACCCTGACAAGAAGCACCAGAAGGAGCCCCCTTCCCTGTGGATGGGCTATGAGCTGCAC  
 euLeuArgTrpGlyLeuThrThrProAspLysLysHisGlnLysGluProProPheLeuTrpMetGlyTyrGluLeuHis  
 220 230  
 CCGACAAGTGGACTGTGCAGCCCATTTGTGCTGCCTGACAAGGACTCCTGGACTGTGAATGACATCCAGAAGCTGGTGGG  
 ProAspLysTrpThrValGlnProIleValLeuProGluLysAspSerTrpThrValAsnAspIleGlnLysLeuValG  
 240 250 260  
 CAAGCTGAAGTGGGCTCCCAAATCTACCTGGCATCAAGGTGAGGCAGCTGTGCAAGCTGCTGAGGGGCACCAAGGCC  
 yLysLeuAsnTrpAlaSerGlnIleTyrProGlyIleLysValArgGlnLeuCysLysLeuLeuArgGlyThrLysAlaL  
 270 280 290

FIGURE 17A

TGACTGAGGTGATCCCCCTGACTGAGGAGGCTGAGCTGGAGCTGGCTGAGAACAGGAGATCCTGAAGGAGCCTGTGCAT  
 EuThrGluVol||eProLeuThrGluGluAlaGluLeuGluLeuAlaGluAsnArgGlu||eLeuLysGluProVolHis  
 300 310

GGGGTGACTATGACCCCTCCAAGGACCTGATGCTGAGATCCAGAAGCAGGGCCAGGGCCAGTGACCTACCAATCTA  
 GlyVolTyrTyrAspProSerLysAspLeu||eAlaGlu||eGlnLysGlnGlyGlnGlyGlnTrpThrTyrGln||eTy  
 320 330 340

CCAGGAGCCCTTCAAGAACCTGAAGACTGGCAAGTATGCCAGGATGAGGGGGGGCCACACCAATGATGTGAAGCAGCTGA  
 rGlnGluProPheLysAsnLeuLysThrGlyLysTyrAlaArgMeLArgGlyAlaHisThrAsnAspVolLysGlnLeuT  
 350 350 370

CTGAGGCTGTGCAGAAGATCACCAGTGGTCCATGTGATCTGGGGCAAGACCCCCAAGTTCAAGCTGCCCATCCAGAAG  
 hrGluAlaVolGlnLys||eThrThrGluSer||eVol||eTrpGlyLysThrProLysPheLysLeuPro||eGlnLys  
 380 390

GAGACCTGGGAGACCTGGTGGACTGAGTACTGGCAGGCCACCTGGATCCCTGAGTGGGAGTTTGTGAACACCCCCCCCCT  
 GluThrTrpGluThrTrpTrpThrGluTyrTrpGlnAlaThrTrp||eProGluTrpGluPheVolAsnThrProProLe  
 400 410 420

GGTGAAGCTGTGGTACCAGCTGGAGAAGGAGCCCATGTGGGGCTGAGACCTTCTATGTGGCTGGGGCTGCCAACAGGG  
 uVolLysLeuTrpTyrGlnLeuGluLysGluPro||eVolGlyAlaGluThrPheTyrVolAlaGlyAlaAlaAsnArgG  
 430 440 450

AGACCAAGCTGGGCAAGGCTGGCTATGTGACCAACAGGGGCAGGCAGAACGGTGGTGAACCTGACTGACACCACCAACCAG  
 luThrLysLeuGlyLysAlaGlyTyrVolThrAsnArgGlyArgGlnLysVolVolThrLeuThrAspThrThrAsnGln  
 460 470

AAGACTGCCCTCCAGGCCATCTACCTGGCCCTCCAGGACTCTGGCCTGGAGGTGAACATTGTGACTGCCCTCCAGTATGC  
 LysThrAlaLeuGlnAla||eTyrLeuAlaLeuGlnAspSerGlyLeuGluVolAsn||eVolThrAlaSerGlnTyrAl  
 480 490 500

CCTGGGCATCATCCAGGCCAGCCTGATCAGTCTGAGTCTGAGCTGGTGAACCAGATCATTGAGCAGCTGATCAAGAAGG  
 oLeuGly||e||eGlnAlaGlnProAspGlnSerGluSerGluLeuVolAsnGln||e||eGluGlnLeu||eLysLysG  
 510 520 530

AGAAGGTGTACCTGGCCTGGGTGCCTGCCACAAGGCATTGGGGCAATGAGCAGGTGGACAAGCTGGTGTCTGCTGGC  
 luLysVolTyrLeuAlaTrpVolProAlaHisLysGly||eGlyGlyAsnGluGlnVolAspLysLeuVolSerAlaGly  
 540 550

ATCAGGAAGGTGCTGTTCCCTGGATGGCATTGACAAGCCCCAGGATGAGCATGACAAGTACCACTCCAACCTGGAGGGCTAT  
 ||eArgLysVolLeuPheLeuAspGly||eAspLysAlaGlnAspGluHisGluLysTyrHisSerAsnTrpArgAlaMe  
 560 570 580

FIGURE 17B

GGCCTCTGACTTCAACCTGCCCTGTGGTGGCTAAGGAGATTGTGCCCTCCTGTGACAACTGCCAGCTGAAGGGGAGG  
 tAlaSerAspPheAsnLeuProProValVolAlaLysGluIleVolAlaSerCysAspLysCysGlnLeuLysGlyGluA  
 590 600 610

CCATGCATGGCAGGTGGACTGCTCCCTGGCATCTGGCAGCTGGCTGCACCCACCTGGAGGGCAAGGTATCCTGGTG  
 lAlaMetHisGlyGlnVolAspCysSerProGlyIleTrpGlnLeuAlaCysThrHisLeuGluGlyLysVolIleLeuVol  
 620 630

GCTGTGCATGTGGCTCCGGCTACATTGAGGCTGAGGTGATCCCTGCTGAGACAGGCCAGGAGACTGCCTACTTCCTGCT  
 AlaVolHisVolAlaSerGlyTyrIleGluAlaGluVolIleProAlaGluThrGlyGlnGluThrAlaTyrPheLeuLe  
 640 650 660

GAAGCTGGCTGGCAGGTGGCTGTGAAGACCATCCACACTGCCAATGGCTCCAATTCACTGGGGCCACAGTGAGGGCTG  
 uLysLeuAlaGlyArgTrpProValLysThrIleHisThrAlaAsnGlySerAsnPheThrGlyAlaThrValArgAlaA  
 670 680 690

CCTGCTGGTGGCTGGCATCAAGCAGGAGTTTGGCATCCCTACAACCCCACTCCACGGGGTGGTGGCTCCATGAAC  
 lAlaCysTrpTrpAlaGlyIleLysGlnGluPheGlyIleProTyrAsnProGlnSerGlnGlyVolVolAlaSerMetAsn  
 700 710

AAGGAGCTGAAGAAGATCATTGGGCAGGTGAGGGACCAGGCTGAGCACCTGAAGACAGCTGTGCAGATGGCTGTGTTCAT  
 LysGluLeuLysLysIleIleGlyGlnVolArgAspGlnAlaGluHisLeuLysThrAlaVolGlnMetAlaVolPheIle  
 720 730 740

CCACAATTCAAGAGGAAGGGGGCATCGGGGGCTACTCGCTGGGGAGAGGATTGTGGACATCATTGCCACAGACATCC  
 eHisAsnPheLysArgLysGlyGlyIleGlyGlyTyrSerAlaGlyGluArgIleVolAspIleIleAlaThrAspIleG  
 750 760 770

AGACCAAGGAGCTCCAGAAGCAGATCACCAAGATCCAGAACTTCAGGCTGTACTACAGGACTCCAGGAACCCCTGTGG  
 lThrLysGluLeuGlnLysGlnIleThrLysIleGlnAsnPheArgValTyrTyrArgAspSerArgAsnProLeuTrp  
 780 790

AAGGGCCCTGCCAAGCTGCTGTGGAAGGGGGAGGGGGCTGTGGTGATCCAGGACAACTCTGACATCAAGGTGGTGGCCAG  
 LysGlyProAlaLysLeuLeuTrpLysGlyGluGlyAlaVolVolIleGlnAspAsnSerAspIleLysVolVolProAr  
 800 810 820

GAGGAAGGCCAAGATCATCAGGACTATGGCAAGCAGATGGCTGGGGATGACTGTGTGGCTCCAGGCAGGATGAGGACT  
 gArgLysAlaLysIleIleArgAspTyrGlyLysGlnMetAlaGlyAspAspCysValAlaSerArgGlnAspGluAspx  
 830 840 850

AAAGCCCGGGCAGATC (SEQ ID NO: 3)  
 Xx Bgll (SEQ ID NO: 4)

FIGURE 17C

[illegible]

**FIGURE 18**

|     |                                                           |      |
|-----|-----------------------------------------------------------|------|
| WT  | - ATG GGT GGC AAG TGG TCA AAA CGT AGT GTG CCT GGA TGG TCT | -42  |
|     |                                                           |      |
| OPT | - ATG GGC GGC AAG TGG TCC AAG AGG TCC GTG CCC GGC TGG TCC |      |
|     | M G G K W S K R S V P G W S                               | -14  |
| WT  | - ACT GTA AGG GAA AGA ATG AGA CGA GCT GAG CCA GCA GCA GAT | -84  |
|     |                                                           |      |
| DPT | - ACC GTG AGG GAG AGG ATG AGG AGG GCC GAG CCC GCC GCC GAC |      |
|     | T V R E R M R R A E P A A D                               | -28  |
| WT  | - AGG GTG AGA CGA ACT GAG CCA GCA GCA GTA GGG GTG GGA GCA | -126 |
|     |                                                           |      |
| OPT | - AGG GTG AGG AGG ACC GAG CCC GCC GCC GTG GGC GTG GGC GCC |      |
|     | R V R R T E P A A V G V G A                               | -42  |
| WT  | - GTA TCT CGA GAC CTG GAA AAA CAT GGA GCA ATC ACA AGT AGC | -168 |
|     |                                                           |      |
| OPT | - GTG TCC AGG GAC CTG GAG AAG CAC GGC GCC ATC ACC TCC TCC |      |
|     | V S R D L E K H G A I T S S                               | -56  |
| WT  | - AAT ACA GCA GCT ACC AAT GCT GAT TGT GCC TGG CTA GAA GCA | -210 |
|     |                                                           |      |
| OPT | - AAC ACC GCC GCC ACC AAC GCC GAC TGC GCC TGG CTG GAG GCC |      |
|     | N T A A T N A D C A W L E A                               | -70  |
| WT  | - CAA GAG GAT GAG GAA GTG GGT TTT CCA GTC AGA CCT CAG GTA | -252 |
|     |                                                           |      |
| OPT | - CAG GAG GAC GAG GAG GTG GGC TTC CCC GTG AGG CCC CAG GTG |      |
|     | Q E D E E V G F P V R P Q V                               | -84  |
| WT  | - CCT TTA AGA CCA ATG ACT TAC AAG GGA GCT GTA GAT CTT AGC | -294 |
|     |                                                           |      |
| OPT | - CCC CTG AGG CCC ATG ACC TAC AAG GGC GCC GTG GAC CTG TCC |      |
|     | P L R P M T Y K G A V D L S                               | -98  |
| WT  | - CAC TTT TTA AAA GAA AAG GGG GGA CTG GAA GGG CTA ATT CAC | -336 |
|     |                                                           |      |
| OPT | - CAC TTC CTG AAG GAG AAG GGC GGC CTG GAG GGC CTG ATC CAC |      |
|     | H F L K E K G G L E G L I H                               | -112 |
| WT  | - TCA CAG AAA AGA CAA GAT ATC CTT GAT CTG TGG GTC TAC CAC | -378 |
|     |                                                           |      |
| OPT | - TCC CAG AAG AGG CAG GAC ATC CTG GAC CTG TGG GTG TAC CAC |      |
|     | S Q K R Q D I L D L W V Y H                               | -126 |
| WT  | - ACA CAA GGC TAC TTC CCT GAT TGG CAG AAC TAC ACA CCA GGG | -420 |
|     |                                                           |      |
| OPT | - ACC CAG GGC TAC TTC CCC GAC TGG CAG AAC TAC ACC CCC GGC |      |
|     | T Q G Y F P D W Q N Y T P G                               | -140 |

FIGURE 19A

|     |                                                              |      |
|-----|--------------------------------------------------------------|------|
| WT  | - CCA GGA ATC AGA TTT CCA TTG ACC TTT GGA TGG TGC TTC AAG    | -462 |
|     |                                                              |      |
| OPT | - CCC GGC ATC AGG TTC CCC CTG ACC TTC GGC TGG TGC TTC AAG    |      |
|     | P G I R F P L T F G W C F K                                  | -154 |
| WT  | - CTA GTA CCA GTT GAG CCA GAA AAG GTA GAA GAG GCC AAT GAA    | -504 |
|     |                                                              |      |
| OPT | - CTG GTG CCC GTG GAG CCC GAG AAG GTG GAG GAG GCC AAC GAG    |      |
|     | L V P V E P E K V E E A N E                                  | -168 |
| WT  | - GGA GAG AAC AAC TGC TTG TTA CAC CCT ATG AGC CAG CAT GGG    | -546 |
|     |                                                              |      |
| OPT | - GGC GAG AAC AAC TGC CTG CTG CAC CCC ATG TCC CAG CAC GGC    |      |
|     | G E N N C L L H P M S Q H G                                  | -182 |
| WT  | - ATA GAG GAC CCG GAG AAG GAA GTG TTA GAG TGG AGG TTT GAC    | -588 |
|     |                                                              |      |
| OPT | - ATC GAG GAC CCC GAG AAG GAG GTG CTG GAG TGG AGG TTC GAC    |      |
|     | I E D P E K E V L E W R F D                                  | -196 |
| WT  | - AGC AAG CTA GCA TTT CAT CAC GTG GCC CGA GAG CTG CAT CCG    | -630 |
|     |                                                              |      |
| OPT | - TCC AAG CTG GCC TTC CAC CAC GTG GCC AGG GAG CTG CAC CCC    |      |
|     | S K L A F H H V A R E L H P                                  | -210 |
| WT  | - GAG TAC TAC AAG GAC TGC TGA (SEQ ID NO:30)                 | -651 |
|     |                                                              |      |
| OPT | - GAG TAC TAC AAG GAC TGC TAA (contained within SEQ ID NO:9) |      |
|     | E Y Y K D C (SEQ ID NO:10)                                   | -216 |

FIGURE 19B

VIJns/nef *PstI* *BglII*  
CATGGGTCCTTTTCGAGTCACCGTCCTTGAAGATCTGCCACC ATG GGC GGC ANG TGG TCC MAG AGG TCC GTG CCC . . . . .  
M G G K W S K R S V P

. . . . . CAC CCC GAG TAC TAC ANG GAC TGC TAA *SrfI* *BglII*  
AGCCCGGGGAGATCTGCTGTGCTTCTAGTTGCCAGC (SEQ ID NO: 38)  
H P E Y Y K D C \* (contained within SEQ ID NO: 10)

VIJns/nef(G2A.LLAA)

*PstI* *BglII*  
CATGGGTCCTTTTCGAGTCACCGTCCTTGAAGATCTGCCACC ATG GGC GGC ANG TGG TCC MAG AGG TCC GTG CCC . . . . .  
M A G K W S K R S V P

. . . . . CAC CCC GAG TAC TAC ANG GAC TGC TAA *SrfI* *BglII*  
AGCCCGGGGAGATCTGCTGTGCTTCTAGTTGCCAGC (SEQ ID NO: 39)  
H P E Y Y K D C \* (contained within SEQ ID NO: 14)

VIJns/tpanef & VIJns/tpanef(LLAA)

*PstI* *BglII*  
CATGGGTCCTTTTCGAGTCACCGTCCTTATATCTAGATCACC ATG GAT GCA ATG MAG AGA GGG CTC TGC TGT GTG  
M D A M K R G L C V

CTG CTG CTG TGT GGA GCA GTC TTC GTT TCG CCC AGC *BglII*  
GAGATCTGCCACC TCC TCC MAG AGG TCC GTG CCC . . . . .  
L L L C G A V F V S P S E I S S K R S V P

. . . . . CAC CCC GAG TAC TAC ANG GAC TGC TAA *SrfI* *BglII*  
AGCCCGGGGAGATCTGCTGTGCTTCTAGTTGCCAGC (SEQ ID NO: 40)  
H P E Y Y K D C \* (contained within SEQ ID NO: 16)

FIGURE 20



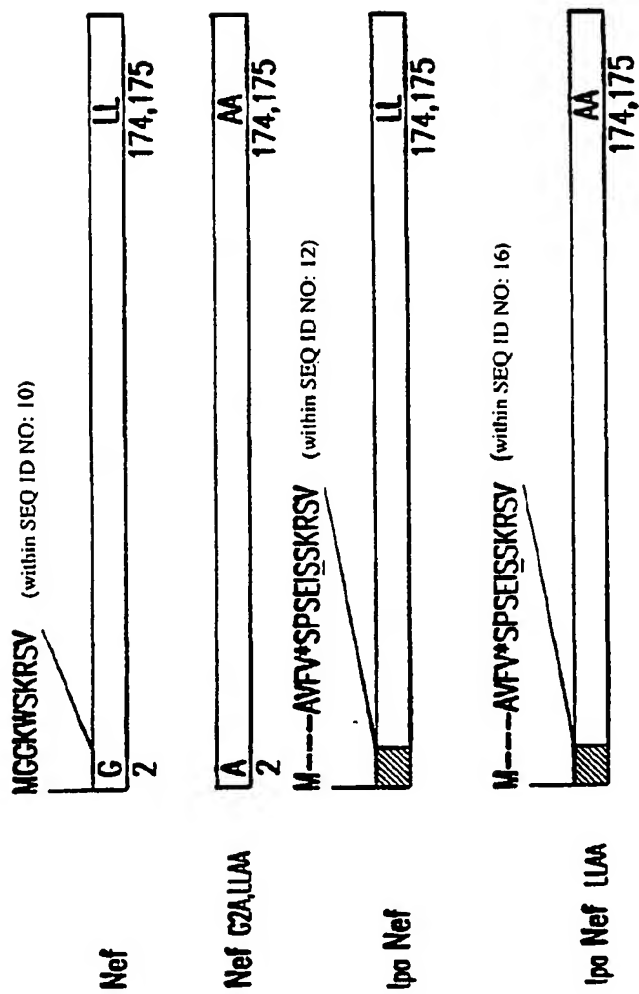


FIGURE 21

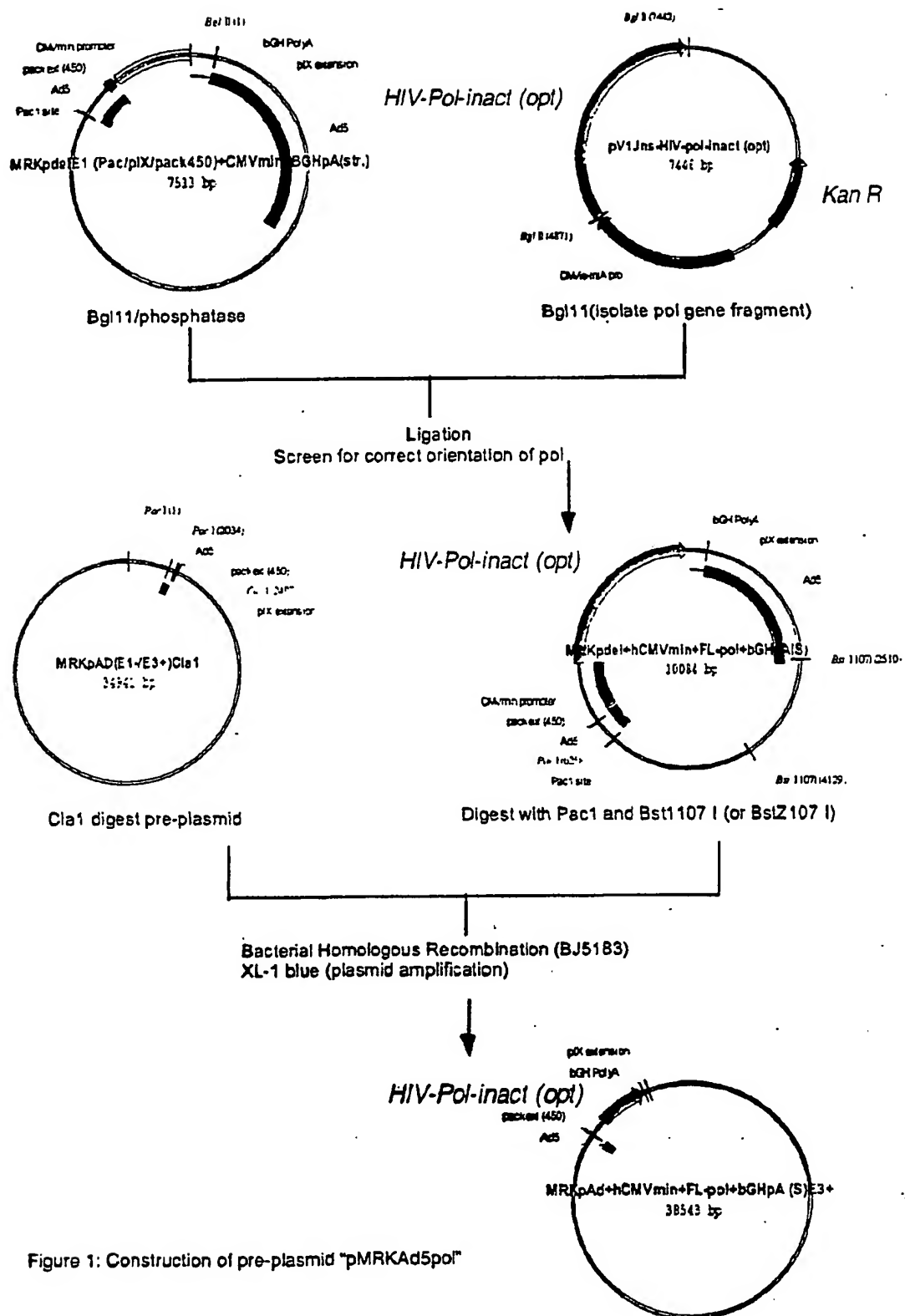


Figure 1: Construction of pre-plasmid "pMRKAd5pol"

FIGURE 22

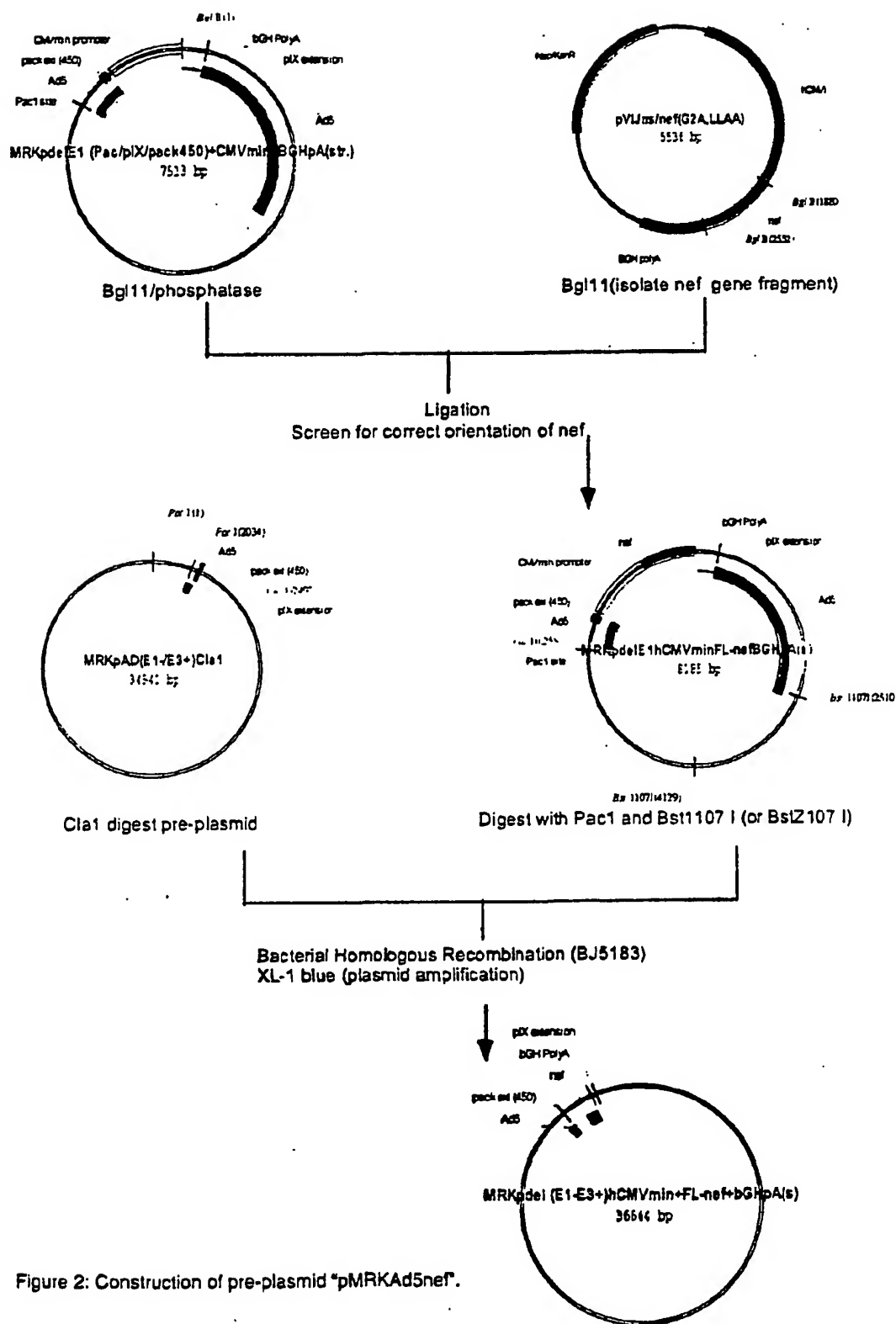
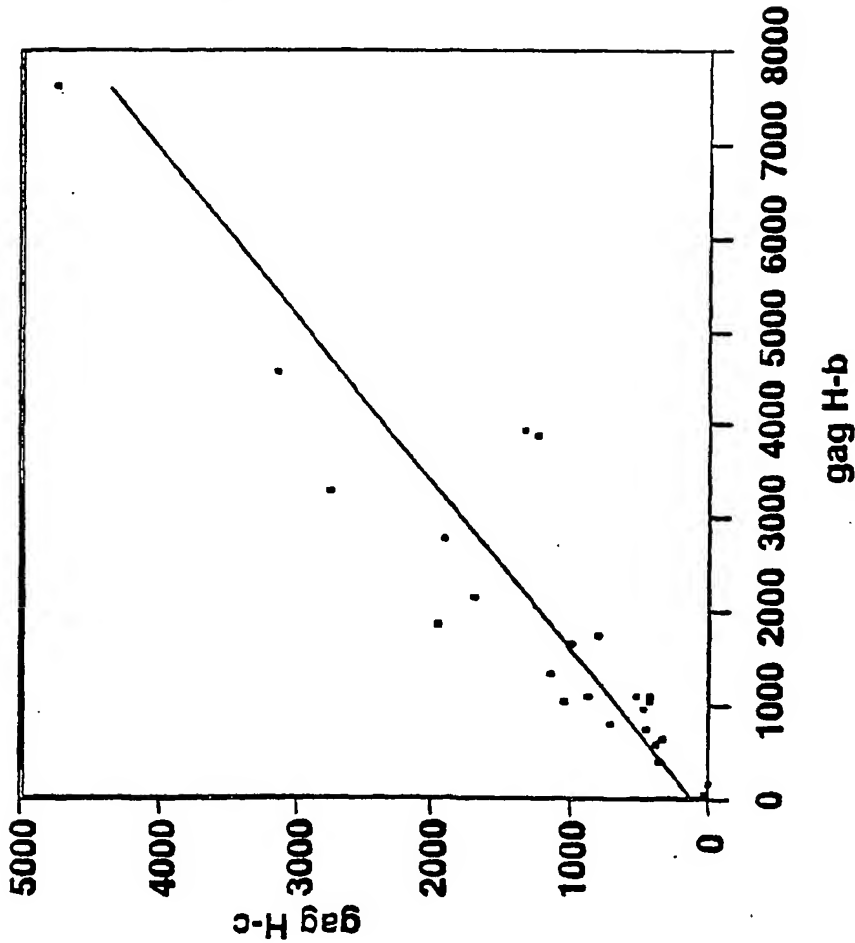


Figure 2: Construction of pre-plasmid "pMRKAd5nef".

FIGURE 23

**Comparison of Clade B vs. Clade C Anti-gag T Cell Responses in Clade B HIV-Infected Subjects**



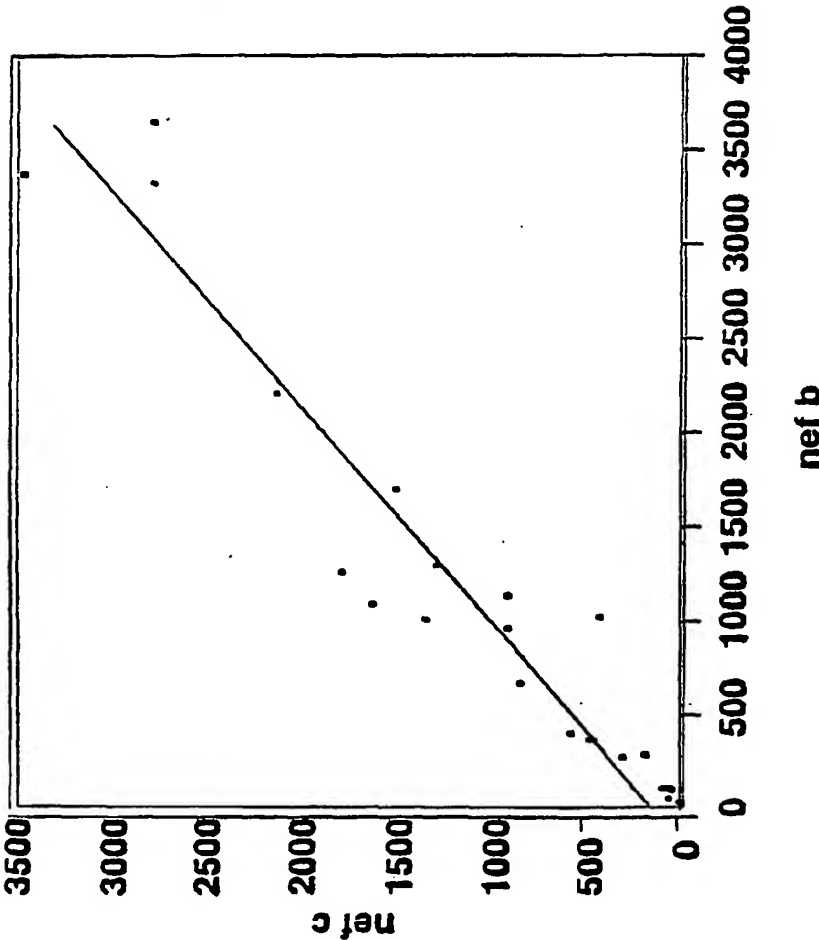
Linear Fit

$$\text{gag H-c} = 111.603 + 0.55866 \text{ gag H-b}$$

Summary of Fit

|                            |          |
|----------------------------|----------|
| RSquare                    | 0.816775 |
| RSquare Adj                | 0.80914  |
| Root Mean Square Error     | 474.9639 |
| Mean of Response           | 1158.115 |
| Observations (or Sum Wgts) | 26       |

# Comparison of Clade B vs. Clade C Anti-nef T Cell Responses in Clade B HIV-Infected Subjects



Summary of Fit

|                            |          |
|----------------------------|----------|
| RSquare                    | 0.91685  |
| RSquare Adj                | 0.91289  |
| Root Mean Square Error     | 289.7718 |
| Mean of Response           | 1096.435 |
| Observations (or Sum Wgts) | 23       |

FIGURE 25

**MRKAd5pol MER1062**  
(MRKAd5 Pre-Adenoviral Vector Containing the IA opt pol Coding Region)

```

1  CATCATCAAT AATATACCTT ATTTTGGATT GAAGCCAATA TGATAATGAG
   GTAGTAGTTA TTATATGGAA TAAACCTAA CTTCCGTTAT ACTATTACTC

51  GGGGTGGAGT TTGTGACGTG GCGCGGGGCG TGGGAACGGG GCGGGTGACG
   CCCCACCTCA AACACTGCAC CGCGCCCCGC ACCCTTGCCC CGCCCACTGC

101 TAGTAGTGTG GCGGAAGTGT GATGTTGCAA GTGTGGCGGA ACACATGTAA
   ATCATCACAC CGCCTTCACA CTACAACGTT CACACCGCCT TGTGTACATT

151 GCGACGGATG TGGCAAAAGT GACGT'TTTTG GTGTGCGCCG GTGTACACAG
   CGCTGCCCTAC ACCGTTTTC A TGCAAAAAC CACACGCGGC CACATGTGTC

201 GAAGTGACAA TTTTCGCGCG GTTTTAGGCG GATGTTGTAG TAAATTTGGG
   CTTCACTGTT AAAAGCGCGC CAAATCCGC CTACAACATC ATTTAAACCC

251 CGTAACCGAG TAAGATTGCG CCATTTTCGC GGGAAACTG AATAAGAGGA
   GCATTGGCTC ATTCTAAACC GGTAAAAGCG CCGTTTGTAC TTATTCTCCT

301 AGTGAAATCT GAATAATTTT GTGTTACTCA TAGCGCGTAA TATTTGTCTA
   TCACTTTAGA CTTATTAAAA CACAATGAGT ATCGCGCATT ATAAACAGAT

351 GGGCCGCGGG GACTTTGACC GTTTACGTGG AGACTCGCCC AGGTGTTTTT
   CCCGGCGCCC CTGAACTGG CAAATGCACC TCTGAGCGGG TCCACAAAAA

401 CTCAGGTGTT TTCCGCGTTC CGGGTCAAAG TTGGCGTTTT ATTATTATAG
   GAGTCCACAA AAGGCACAAG GCCCAGTTTC AACCACAAA TAATAATATC

451 GCGGCCGCGA TCCATTGCAT ACGTTGTATC CATATCATAA TATGTACATT
   CGCCGGCGCT AGGTAACGTA TGCAACATAG GTATAGTATT ATACATGTAA

501 TATATTGGCT CATGTCCAAC ATTACCGCCA TGTGACATT GATTATTGAC
   ATATAACCGA GTACAGGTG TAATGGCGGT ACAAAGTAA CTAATAACTG

551 TAGTTATTAA TAGTAATCAA TTACGGGGTC ATTAGTTCAT AGCCCATATA
   ATCAATAATT ATCATTAGTT AATGCCCCAG TAATCAAGTA TCGGGTATAT

601 TGGAGTTCCT CGTTACATAA CTTACGTAA ATGGCCCGCC TGGCTGACCG
   ACCTCAAGGC GCAATGTATT GAATGCCATT TACCGGGCGG ACCGACTGGC

651 CCCAACGACC CCCGCCCAT TACGTCAATA ATGACGTATG TTCCCATAGT
   GGGTTGCTGG GGGCGGGTAA CTGCAGTTAT TACTGCATAC AAGGGTATCA

701 AACGCCAATA GGGACTTTC ATTGACGTCA ATGGGTGGAG TATTTACGGT
   TTGCGGTTAT CCCTGAAAGG TAACTGCAGT TACCCACCTC ATAAATGCCA

751 AAAC TGCCCCA CTTGGCAGTA CATCAAGTGT ATCATATGCC AAGTACGCC
   TTTGACGGGT GAACCGTCAT GTAGTTCACA TAGTATACGG TTCATGCGGG

801 CCTATTGACG TCAATGACGG TAAATGGCCC GCCTGGCATT ATGCCAGTA
   GGATAACTGC AGTTACTGCC ATTTACCGGG CGGACCGTAA TACGGGTATC

851 CATGACCTTA TGGGACTTTC CTACTTGGCA GTACATCTAC GTATTAGTCA
   GTACTGGAAT ACCCTGAAAG GATGAACCGT CATGTAGATG CATAATCAGT

```

*Figure 26A*

901 TCGCTATTAC CCGGTGATG CGGTTTTGGC AGTACATCAA TGGGCCCA  
 AGCGATAATG GTACCACTAC GCCAAAACCG TCATGTAGTT ACCCGCACCT  
 951 TAGCGGTTTG ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA  
 ATCGCCAAAC TGAGTGCCCC TAAAGGTCA GAGGTGGGT AACTGCAGTT  
 1001 TGGGAGTTTG TTTTGGCACC AAAATCAACG GGACTTTCCA AAATGTCGTA  
 ACCCTCAAAC AAAACCGTGG TTTTAGTTGC CCTGAAAGGT TTTACAGCAT  
 1051 ACAACTCCGC CCCATTGACG CAAATGGGCG GTAGGCGTGT ACGGTGGGAG  
 TGTGTAGGCG GGGTAACTGC GTTTACCCGC CATCCGCACA TGCCACCCTC  
 1101 GTCTATATAA GCAGAGCTCG TTTAGTGAAC CGTCAGATCG CCTGGAGACG  
 CAGATATATT CGTCTCGAGC AAATCACTTG GCAGTCTAGC GGACCTCTGC  
 1151 CCATCCACGC TGTTTTGACC TCCATAGAAG ACACCGGGAC CGATCCAGCC  
 GGTAGGTGCG ACAAACCTGG AGGTATCTTC TGTGGCCCTG GCTAGGTGCG  
 1201 TCCGCGGCCG GGAACGGTGC ATTGGAACGC GGATTCCCCG TGCCAAGAGT  
 AGGCGCCGGC CCTTGCCACG TAACCTTGCG CCTAAGGGG ACGGTTCTCA  
 1251 GAGATCTACC ATGGCCCCCA TCTCCCCCAT TGAGACTGTG CCTGTGAAGC  
 CTCTAGATGG TACCGGGGGT AGAGGGGGTA ACTCTGACAC GGACACTTCG  
 1301 TGAAGCCTGG CATGGATGGC CCCAAGGTGA AGCAGTGGCC CCTGACTGAG  
 ACTTCGGACC GTACCTACCG GGGTTCCACT TCGTCACCGG GGACTGACTC  
 1351 GAGAAGATCA AGGCCCTGGT GGAAATCTGC ACTGAGATGG AGAAGGAGGG  
 CTCTTCTAGT TCCGGGACCA CCTTTAGACG TGACTCTACC TCTTCCTCCC  
 1401 CAAATCTCC AAGATTGGCC CCGAGAACCC CTACAACACC CCTGTGTTTG  
 GTTTTAGAGG TTCTAACCGG GGCTCTTGGG GATGTTGTGG GGACACAAAC  
 1451 CCATCAAGAA GAAGGACTCC ACCAAGTGGA GGAAGCTGGT GGACTTCAGG  
 GGTAGTTCTT CTTCTGAGG TGGTTCACCT CCTTCGACCA CCTGAAGTCC  
 1501 GAGCTGAACA AGAGGACCCA GGACTTCTGG GAGGTGCAGC TGGGCATCCC  
 CTCGACTTGT TCTCCTGGGT CCTGAAGACC CTCCACGTCG ACCCGTAGGG  
 1551 CCACCCCGCT GGCCTGAAGA AGAAGAAGTC TGTGACTGTG CTGGCTGTGG  
 GGTGGGGCGA CCGGACTTCT TCTTCTCAG AACTGACAC GACCGACACC  
 1601 GGGATGCCA CTTCTCTGTG CCCCTGGATG AGGACTTCAG GAAGTACACT  
 CCCTACGGAT GAAGAGACAC GGGGACCTAC TCCTGAAGTC CTTCATGTGA  
 1651 GCCTTCACCA TCCCCTCCAT CAACAATGAG ACCCCTGGCA TCAGGTACCA  
 CGGAAGTGGT AGGGGAGGTA GTTGTACTC TGGGGACCGT AGTCCATGGT  
 1701 GTACAATGTG CTGCCCCAGG GCTGGAAGGG CTCCCCTGCC ATCTTCCAGT  
 CATGTTACAC GACGGGGTCC CGACCTTCCC GAGGGGACGG TAGAAGGTCA  
 1751 CCTCCATGAC CAAGATCCTG GAGCCCTTCA GGAAGCAGAA CCCTGACATT  
 GGAGGTACTG GTTCTAGGAC CTCGGGAAGT CCTTCGTCTT GGGACTGTAA  
 1801 GTGATCTACC AGTACATGGC TGCCCTGTAT GTGGGCTCTG ACCTGGAGAT  
 CACTAGATGG TCATGTACCG ACGGGACATA CACCCGAGAC TGGACCTCTA

Figure 26 B

1851 TGGGCAGCAC A CCAAGA TTGAGGAGCT GAGGCAGCAC CTGCTG T  
 ACCCGTCGTG TCTTGGTTCT AACTCCTCGA CTCCGTCGTG GACGACTCCA  
 1901 GGGGCCTGAC CACCCCTGAC AAGAAGCACC AGAAGGAGCC CCCCTTCCTG  
 CCCC GGACTG GTGGGGACTG TTCTTCGTGG TCTTCCTCGG GGGGAAGGAC  
 1951 TGGATGGGCT ATGAGCTGCA CCCCAGCAAG TGGACTGTGC AGCCCATTTG  
 ACCTACCCGA TACTCGACGT GGGGCTGTTT ACCTGACACG TCGGGTAACA  
 2001 GCTGCCTGAG AAGGACTCCT GGACTGTGAA TGACATCCAG AAGCTGGTGG  
 CGACGGACTC TTCCTGAGGA CCTGACACTT ACTGTAGGTC TTCGACCACC  
 2051 GCAAGCTGAA CTGGGCCTCC CAAATCTACC CTGGCATCAA GGTGAGGCAG  
 CGTTCGACTT GACCCGGAGG GTTTAGATGG GACCGTAGTT CCACTCCGTC  
 2101 CTGTGCAAGC TGCTGAGGGG CACCAAGGCC CTGACTGAGG TGATCCCCCT  
 GACACGTTCTG ACGACTCCCC GTGGTTCCGG GACTGACTCC ACTAGGGGGA  
 2151 GACTGAGGAG GCTGAGCTGG AGCTGGCTGA GAACAGGGAG ATCCTGAAGG  
 CTGACTCCTC CGACTCGACC TCGACCGACT CTTGTCCCTC TAGGACTTCC  
 2201 AGCCTGTGCA TGGGGTGAC TATGACCCCT CCAAGGACCT GATTGCTGAG  
 TCGGACACGT ACCCCACATG ATACTGGGGA GGTTCCTGGA CTAACGACTC  
 2251 ATCCAGAAGC AGGGCCAGGG CCAGTGGACC TACCAAATCT ACCAGGAGCC  
 TAGGTCTTCG TCCCGGTCCC GGTCACTTGG ATGGTTTAGA TGGTCTCGG  
 2301 CTTCAAGAAC CTGAAGACTG GCAAGTATGC CAGGATGAGG GGGGCCCCA  
 GAAGTTCTTG GACTTCTGAC CGTTCATACG GTCTTACTCC CCCCCGGTGT  
 2351 CCAATGATGT GAAGCAGCTG ACTGAGGCTG TGCAGAAGAT CACCACTGAG  
 GGTACTACA CTTCTGTCGAC TGACTCCGAC ACGTCTTCTA GTGGTGACTC  
 2401 TCCATTGTGA TCTGGGGCAA GACCCCCAAG TTCAAGCTGC CCATCCAGAA  
 AGGTAACACT AGACCCCGTT CTGGGGGTTT AAGTTCGACG GGTAGGTCTT  
 2451 GGAGACCTGG GAGACCTGGT GGACTGAGTA CTGGCAGGCC ACCTGGATCC  
 CCTCTGGACC CTCTGGACCA CCTGACTCAT GACCGTCCGG TGGACCTAGG  
 2501 CTGAGTGGGA GTTTGTGAAC ACCCCCCCCC TGGTGAAGCT GTGGTACCAG  
 GACTCACCCCT CAAACACTTG TGGGGGGGGG ACCACTTCGA CACCATGGTC  
 2551 CTGGAGAAGG AGCCCATTTGT GGGGGCTGAG ACCTTCTATG TGGCTGGGGC  
 GACCTCTTCC TCGGGTAACA CCCCCGACTC TGAAGATAC ACCGACCCCG  
 2601 TGCCAACAGG GAGACCAAGC TGGGCAAGGC TGGCTATGTG ACCAACAGGG  
 ACGGTTGTCC CTCTGGTTCTG ACCCGTTCCG ACCGATACAC TGGTTGTCCC  
 2651 GCAGGCAGAA GGTGGTGACC CTGACTGACA CCACCAACCA GAAGACTGCC  
 CGTCCGTCTT CCACCACTGG GACTGACTGT GGTGGTTGGT CTTCTGACGG  
 2701 CTCCAGGCCA TCTACCTGGC CCTCCAGGAC TCTGGCCTGG AGGTGAACAT  
 GAGGTCCGGT AGATGGACCG GGAGGTCCTG AGACCGGACC TCCACTTGTA  
 2751 TGTGACTGCC TCCCAGTATG CCCTGGGCAT CATCCAGGCC CAGCCTGATC  
 AACTGACGG AGGGTCATAC GGGACCCGTA GTAGGTCCGG GTCGGACTAG

Figure 26C



2801 AGTCTGAGTC TCTGGTG AACCAGATCA TTGAGCAGCT GATCAAG  
 TCAGACTCAG ACTCGACCAC TTGGTCTAGT AACTCGTCGA CTAGTCTTC  
 2851 GAGAAGGTGT ACCTGGCCTG GGTGCCTGCC CACAAGGGCA TTGGGGGCAA  
 CTCTTCACAC TGGACCGGAC CCACGGACGG GTGTTCCCGT AACCCCGTT  
 2901 TGAGCAGGTG GACAAGCTGG TGTCTGCTGG CATCAGGAAG GTGCTGTTC  
 ACTCGTCCAC CTGTTTCGACC ACAGACGACC GTAGTCCTTC CACGACAAGG  
 2951 TGGATGGCAT TGACAAGGCC CAGGATGAGC ATGAGAAGTA CCACTCCAAC  
 ACCTACCGTA ACTGTTCCGG GTCTACTCG TACTCTTCAT GGTGAGGTG  
 3001 TGGAGGGCTA TGGCCTCTGA CTTCAACCTG CCCCCTGTGG TGGCTAAGGA  
 ACCTCCCGAT ACCCGAGACT GAAGTTGGAC GGGGGACACC ACCGATTCTT  
 3051 GATTGTGGCC TCCTGTGACA AGTGCCAGCT GAAGGGGGAG GCCATGCATG  
 CTAACACCGG AGGACACTGT TCACGGTCGA CTTCCCCCTC CGGTACGTAC  
 3101 GGCAGGTGGA CTGCTCCCTT GGCATCTGGC AGCTGGCCTG CACCACCTG  
 CCGTCCACCT GACGAGGGGA CCGTAGACCG TCGACCGGAC GTGGGTGGAC  
 3151 GAGGGCAAGG TGATCCTGGT GGCTGTGCAT GTGGCCTCCG GCTACATTGA  
 CTCCCGTTCC ACTAGGACCA CCGACACGTA CACCGGAGGC CGATGTAAC  
 3201 GGCTGAGGTG ATCCCTGCTG AGACAGGCCA GGAGACTGCC TACTTCCTGC  
 CCGACTCCAC TAGGGACGAC TCTGTCCGGT CCTCTGACGG ATGAAGGACG  
 3251 TGAAGCTGGC TGGCAGGTGG CCTGTGAAGA CCATCCACAC TGCCAATGGC  
 ACTTCGACCG ACCGTCCACC GGACACTTCT GGTAGGTGTG ACGGTTACCG  
 3301 TCCAACCTCA CTGGGGCCAC AGTGAGGGCT GCCTGTGCTG GGGCTGGCAT  
 AGGTTGAAGT GACCCCGGTG TCACTCCCGA CGGACGACCA CCCGACCGTA  
 3351 CAAGCAGGAG TTTGGCATCC CCTACAACCC CCAGTCCCAG GGGGTGGTGG  
 GTTCGTCTTC AAACCGTAGG GGATGTTGGG GGTGAGGGTC CCCCACCACC  
 3401 CCTCCATGAA CAAGGAGCTG AAGAAGATCA TTGGGCAGGT GAGGGACCAG  
 GGAGGTACTT GTTCCTCGAC TTCTTCTAGT AACCCGTCCA CTCCCTGGTC  
 3451 GCTGAGCACC TGAAGACAGC TGTGCAGATG GCTGTGTTCA TCCACAACCT  
 CGACTCGTGG ACTTCTGTG ACACGTCTAC CGACACAAGT AGGTGTTGAA  
 3501 CAAGAGGAAG GGGGGCATCG GGGGCTACTC CGCTGGGGAG AGGATTGTGG  
 GTTCTCCTTC CCCCCTAGC CCCCCTAGG GCGACCCCTC TCCTAACACC  
 3551 ACATCATTCG CACAGACATC CAGACCAAGG AGCTCCAGAA GCAGATCACC  
 TGTAGTAACG GTGTCTGTAG GTCTGGTTCC TCGAGGTCTT CGTCTAGTGG  
 3601 AAGATCCAGA ACTTCAGGGT GTACTACAGG GACTCCAGGA ACCCCCTGTG  
 TTCTAGGTCT TGAAGTCCCA CATGATGTCC CTGAGGTCTT TGGGGGACAC  
 3651 GAAGGGCCCT GCCAAGCTGC TGTGGAAGGG GGAGGGGGCT GTGGTGATCC  
 CTTCCTCGGA CGGTTCGACG ACACCTTCCC CCTCCCCGA CACCCTAGG  
 3701 AGGACAATC TGACATCAAG GTGGTGCCCA GGAGGAAGGC CAAGATCATC  
 TCCTGTTGAG ACTGTAGTTC CACCACGGGT CCTCCTTCCG GTTCTAGTAG

Figure 26 D

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3751 AGGGACTATG GAGCAGAT GGCTGGGGAT GACTGTGTGG CCTCCATCA
    TCCCTGATAC CTTTCGTCTA CCGACCCCTA CTGACACACC GGAGGTGGT
3801 GGATGAGGAC TAAAGCCCGG GCAGATCTGC TGTGCCTTCT AGTTGCCAGC
    CCTACTCCTG ATTTCCGGCC CGTCTAGACG ACACGGAAGA TCAACGGTCG
3851 CATCTGTTGT TTGCCCCCTCC CCCGTGCCTT CCTTGACCCT GGAAGGTGCC
    GTAGACAACA AACGGGGAGG GGGCACGGAA GGAACGGGA CCTTCCACGG
3901 ACTCCCCTG TCCTTTCCTA ATAAAATGAG GAAATTGCAT CGCATTTGTCT
    TGAGGGTGAC AGGAAAGGAT TATTTTACTC CTTTAACGTA GCGTAACAGA
3951 GAGTAGGTGT CATTCTATTC TGGGGGGTGG GGTGGGGCAG GACAGCAAGG
    CTCATCCACA GTAAGATAAG ACCCCCCACC CCACCCCGTC CTGTGCTTCC
4001 GGGAGGATTG GGAAGACAAT AGCAGGCATG CTGGGGATGC GGTGGGCTCT
    CCCTCCTAAC CCTTCTGTTA TCGTCCGTAC GACCCCTACG CCACCCGAGA
4051 ATGGCCGATC GGC CGCCGT ACTGAAATGT GTGGGCGTGG CTTAAGGGTG
    TACCGGCTAG CCGCGCGGCA TGACTTTACA CACCCGCACC GAATTTCCAC
4101 GGAAAGAATA TATAAGGTGG GGGTCTTATG TAGTTTGTG TCTGTTTTCG
    CCTTCTTAT ATATTCCACC CCCAGAATAC ATCAAAACAT AGACAAAACG
4151 AGCAGCCGCC GCCGCCATGA GCACCAACTC GTTTGATGGA AGCATTGTGA
    TCGTCGGCGG CGCGGGTACT CGTGGTTGAG CAAACTACCT TCGTAACACT
4201 GCTCATATTT GACAACGCGC ATGCCCCCAT GGGCCGGGGT GCGTCAGAAT
    CGAGTATAAA CTGTTGCGCG TACGGGGGTA CCCGGCCCCA CGCAGTCTTA
4251 GTGATGGGCT CCAGCATTGA TGGTCGCCCC GTCCTGCCCG CAAACTCTAC
    CACTACCCGA GGTGCTAACT ACCAGCGGGG CAGGACGGGC GTTTGAGATG
4301 TACCTTGACC TACGAGACCG TGTCTGGAAC GCCGTGGAG ACTGCAGCCT
    ATGGAACGAG ATGCTCTGGC ACAGACCTTG CGGCAACCTC TGACGTCGGA
4351 CCGCCGCCGC TTCAGCCGCT GCAGCCACCG CCCGCGGGAT TGTGACTGAC
    GCGCGCGGCG AAGTCGGCGA CGTCGTTGGC GGGCGCCCTA ACACTGACTG
4401 TTTGCTTTCC TGAGCCCGCT TGCAAACAGT GCAGCTTCCC GTTCATCCGC
    AAACGAAAGG ACTCGGGCGA ACGTTGTCA CGTCGAAGGG CAAGTAGGCG
4451 CCGCGATGAC AAGTTGACGG CTCTTTTGGC ACAATTGGAT TCTTTGACCC
    GCGGCTACTG TTCAACTGCC GAGAAAACCG TGTTAACCTA AGAAACTGGG
4501 GGGAACTTAA TGTGCTTCT CAGCAGCTGT TGGATCTGCG CCAGCAGGTT
    CCCTTGAATT ACAGCAAAGA GTCGTCGACA ACCTAGACGC GGTGCTCCAA
4551 TCTGCCCTGA AGGCTTCCTC CCCTCCCAAT GCGGTTTAAA ACATAAATAA
    AGACGGGACT TCCGAAGGAG GGGAGGGTTA CGCCAAATTT TGTATTTATT
4601 AAAACCAGAC TCTGTTTGGG TTTGGATCAA GCAAGTGTCT TGCTGTCTTT
    TTTTGGTCTG AGACAAACCT AAACCTAGTT CGTTCACAGA ACGACAGAAA
4651 ATTTAGGGGT TTTGCGCGCG CGGTAGGCCC GGGACCAGCG GTCTCGGTGCG
    TAAATCCCCA AAACGCGCGC GCCATCCGGG CCCTGGTTCG CAGAGCCAGC

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Figure 26E

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4701 TTGAGGGTCC TGTGTATTTT TTCCAGGACG TGGTAAAGGT GACTCTGAT
AACTCCCAGG AATAAAAA AAGGTCCTGC ACCATTTCCT CTGAGAA
4751 GTTCAGATAC ATGGGCATAA GCCCGTCTCT GGGGTGGAGG TAGCACCCT
CAAGTCTATG TACCCGTATT CGGGCAGAGA CCCCACCTCC ATCGTGGTGA
4801 GCAGAGCTTC ATGCTGCGGG GTGGTGTGT AGATGATCCA GTCGTAGCAG
CGTCTCGAAG TACGACGCCC CACCACAACA TCTACTAGGT CAGCATCGTC
4851 GAGCGCTGGG CGTGGTGCCT AAAAATGTCT TTCAGTAGCA AGCTGATTGC
CTCGCGACCC GCACCACGGA TTTTACAGA AAGTCATCGT TCGACTAACG
4901 CAGGGGCAGG CCCTTGGTGT AAGTGTTCAC AAAGCGGTTA AGCTGGGATG
GTCCTCCGTC GGAACACACA TTCACAAATG TTTCCGCAAT TCGACCTAC
4951 GGTGCATACG TGGGGATATG AGATGCATCT TGGACTGTAT TTTTAGGTTG
CCACGTATGC ACCCTTATAC TCTACGTAGA ACCTGACATA AAAATCCAAC
5001 GCTATGTTCC CAGCCATATC CCTCCGGGGA TTCATGTTGT GCAGAACCCAC
CGATACAAGG GTCGGTATAG GGAGGCCCTT AAGTACAACA CGTCTTGGTG
5051 CAGCACAGTG TATCCGGTGC ACTTGGGAAA TTTGTCTATG AGCTTAGAAG
GTCGTGTAC ATAGGCCACG TGAACCTTTT AAACAGTACA TCGAATCTTC
5101 GAAATGCGTG GAAGAACTTG GAGACGCCCT TGTGACCTCC AAGATTTTCC
CTTTACGCAC CTCTTGAAC CTCTGCGGGA AACTGAGG TTCTAAAAGG
5151 ATGCATTCGT CCATAATGAT GGCAATGGGC CCACGGGCGG CGGCCTGGGC
TACGTAAGCA GGTATTACTA CCGTTACCCG GGTGCCCGCC GCCGGACCCG
5201 GAAGATATTT CTGGGATCAC TAACGTCATA GTTGTGTTCC AGGATGAGAT
CTTCTATAAA GACCTTAGTG ATTGCAGTAT CAACACAAGG TCCTACTCTA
5251 CGTCATAGGC CATTTTTACA AAGCGCGGGC GGAGGGTGCC AGACTGCGGT
GCAGTATCCG GTAAAAATGT TTCGCGCCCG CCTCCACGG TCTGACGCCA
5301 ATAATGGTTC CATCCGGCCC AGGGCGTAG TTACCTCAC AGATTTGCAT
TATTACCAAG GTAGGCCGGG TCCCGCATC AATGGGAGTG TCTAAACGTA
5351 TTCCACGCT TTGAGTTCAG ATGGGGGGAT CATGTCTACC TCGGGGGCGA
AAGGGTGCGA AACTCAAGTC TACCCCTTA GTACAGATGG ACGCCCGCT
5401 TGAAGAAAAC GGTTTCCGGG GTAGGGGAGA TCAGCTGGGA AGAAAGCAGG
ACTTCTTTTG CCAAAGGCC CATCCCCTCT AGTCGACCCT TCTTTCGTCC
5451 TTCCTGAGCA GCTGCGACTT ACCGCAGCCG GTGGGCCCGT AAATCACACC
AAGGACTCGT CGACGCTGAA TGGCGTCGGC CACCCGGGCA TTTAGTGTGG
5501 TATTACCGGC TGCAACTGGT AGTTAAGAGA GCTGCAGCTG CCGTCATCCC
ATAATGGCCG ACGTTGACCA TCAATCTCT CGACGTCGAC GGCAGTAGGG
5551 TGAGCAGGGG GGCCACTTCG TTAAGCATGT CCCTGACTCG CATGTTTTCC
ACTCGTCCCC CCGGTGAAGC AATTCGTACA GGGACTGAGC GTACAAAAGG
5601 CTGACCAAAT CCGCCAGAAG GCGCTCGCCG CCCAGCGATA GCAGTCTTGG
GACTGGTTTA GCGGCTCTTC CGCGAGCGGC GGGTCGCTAT CGTCAAGAAC

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Figure 26 F

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5651 CAAGGAAGCA AATTTTTCA ACGGTTTGAG ACCGTCCGCC GTAGGCATCC
      GTTCCTTCGT TTCAAAAAGT TGCCAAACTC TGGCAGGCGG CATCCGTACG

5701 TTTTGAGCGT TTGACCAAGC AGTTCCAGGC GGTCACACAG CTCGGTCACC
      AAAACTCGCA AACTGGTTCG TCAAGGTCCG CCAGGGTGTC GAGCCAGTGG

5751 TGCTCTACGG CATCTCGATC CAGCATATCT CCTCGTTTCG CGGGTTGGGG
      ACGAGATGCC GTAGAGCTAG GTCGTATAGA GGAGCAAAGC GCCCAACCCC

5801 CGGCTTTCGC TGTACGGCAG TAGTCGGTGC TCGTCCAGAC GGGCCAGGGT
      GCCGAAAGCG ACATGCCGTC ATCAGCCACG AGCAGGTCTG CCCGGTCCCA

5851 CATGTCTTTC CACGGGCGCA GGGTCCTCGT CAGCGTAGTC TGGGTCACGG
      GTACAGAAAG GTGCCCGCGT CCCAGGAGCA GTCGCATCAG ACCCAGTGCC

5901 TGAAGGGGTG CGCTCCGGGC TCGCGCTGG CCAGGGTGCG CTTGAGGCTG
      ACTTCCCCAC GCGAGGCCCG ACGCGCGACC GGTCCACGCG GAACTCCGAC

5951 GTCCTGCTGG TGCTGAAGCG CTGCCGCTCT TCGCCCTGCG CGTCGGCCAG
      CAGGACGACC ACGACTTCGC GACGGCCAGA AGCGGGACGC GCAGCCGGTC

6001 GTAGCATTTG ACCATGGTGT CATAGTCCAG CCCCTCCGCG GCGTGGCCCT
      CATCGTAAAC TGGTACCACA GTATCAGGTC GGGGAGGCGC CGCACCGGGA

6051 TGGCGCGCAG CTTGCCCTTG GAGGAGGCGC CGCACGAGGG GCAGTGCAGA
      ACCGCGCGTC GAACGGGAAC CTCCTCCGCG GCGTGCTCCC CGTCACGTCT

6101 CTTTTGAGGG CGTAGAGCTT GGGCGCGAGA AATACCGATT CCGGGGAGTA
      GAAAACTCCC GCATCTCGAA CCCGCGCTCT TTATGGCTAA GGCCCTCAT

6151 GGCATCCGCG CCGCAGGCCC CGCAGACGGT CTCGCATTCC ACGAGCCAGG
      CCGTAGGCGC GCGCTCCGGG GCGTCTGCCA GAGCGTAAGG TGCTCGGTCC

6201 TGAGCTCTGG CCGTTCGGGG TCAAAAACCA GGTTTCCCCC ATGCTTTTTG
      ACTCGAGACC GGCAAGCCCC AGTTTTTGGT CCAAAGGGGG TACGAAAAAC

6251 ATGCGTTTCT TACCTCTGGT TTCCATGAGC CGGTGTCCAC GCTCGGTGAC
      TACGCAAAGA ATGGAGACCA AAGGTACTCG GCCACAGGTG CGAGCCACTG

6301 GAAAAGGCTG TCCGTGTCCC CGTATACAGA CTTGAGAGGC CTGTCTCGA
      CTTTTCGGAC AGGCACAGGG GCATATGTCT GAACTCTCCG GACAGGAGCT

6351 GCGGTGTTCC GCGGTCTTCC TCGTATAGAA ACTCGGACCA CTCTGAGACA
      CGCCACAAGG CGCCAGGAGG AGCATATCTT TGAGCCTGGT GAGACTCTGT

6401 AAGGCTCGCG TCCAGGCCAG CACGAAGGAG GCTAAGTGGG AGGGGTAGCG
      TTCCGAGCGC AGGTCCGGTC GTGCTTCCTC CGATTACCC TCCCCATCGC

6451 GTCGTTGTCC ACTAGGGGGT CCACTCGCTC CAGGGTGTGA AGACACATGT
      CAGCAACAGG TGATCCCCCA GGTGAGCGAG GTCCCACT TCTGTGTACA

6501 CGCCCTCTTC GGCATCAAGG AAGGTGATTG GTTTGTAGGT GTAGGCCACG
      GCGGGAGAAG CCGTAGTTCC TTCCACTAAC CAAACATCCA CATCCGGTGC

6551 TGACCGGGTG TTCCTGAAGG GGGGCTATAA AAGGGGTGCG GGGCGCGTTC
      ACTGGCCAC AAGGACTTCC CCCCATATT TTCCCCACC CCCGCGCAAG

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Figure 266

6601 GTCCTCACTC TCTTCCGCAT CGCTGTCTGC CAGGGCCAGG TGTGCTGTG  
CAGGAGTGAG AAGGCGTA GCGACAGACG CTCCCGGTG ACAACGAC

6651 AGTACTCCCT CTGAAAAGCG GGCATGACTT CTGCGCTAAG ATTGTCAATT  
TCATGAGGGA GACTTTTCGC CCGTACTGAA GACGCGATT TAACAGTCAA

6701 TCCAAAACG AGGAGGATTT GATATTCACC TGGCCCGCGG TGATGCCTTT  
AGGTTTTTGC TCCTCCTAAA CTATAAGTGG ACCGGGCGCC ACTACGGAAA

6751 GAGGGTGGCC GCATCCATCT GGTGAGAAAA GACAATCTTT TTGTTGTCAA  
CTCCCAACCG CGTAGGTAGA CCAGTCTTTT CTGTTAGAAA AACACAGTT

6801 GCTTGGTGGC AAACGACCCG TAGAGGGCGT TGGACAGCAA CTGGCGATG  
CGAACACCG TTTGCTGGGC ATCTCCGCA ACCTGTCGT GAACCGCTAC

6851 GAGCGCAGGG TTTGTTTTT GTGCGATCG GCGCGCTCCT TGGCCGCGAT  
CTGCGTCCC AAACCAAAA CAGCGTAGC CCGCGAGGA ACCGGCGCTA

6901 GTTTAGCTGC ACGTATTGCG GCGCAACGCA CCGCCATTG GGAAGACGG  
CAATCGACG TGCATAAGCG CGCGTTGCGT GCGGTAAGC CCTTCTGCC

6951 TGGTGCCTC GTCCGGCACC AGGTGCACGC GCCAACCGCG GTTGTGCAGG  
ACCACGCGAG CAGCCCGTGG TCCACGTGCG CGGTGGCGC CAACACGTCC

7001 GTGACAAGGT CAACGCTGGT GGCTACCTCT CCGCGTAGGC GCTCGTTGGT  
CACTGTTCCA GTTGCACCA CCGATGGAGA GCGCATCCG CGAGCAACCA

7051 CCAGCAGAGG CGGCCGCCCT TGCAGAGCA GAATGGCGGT AGGGGTCTA  
GGTGTCTCC GCCGCGGGA ACGCGCTCGT CTACCGCA TCCCCAGAT

7101 GCTGCGTCTC GTCCGGGGG TCTGCGTCCA CGTAAAGAC CCCGGCAGC  
CGACGAGAG CAGGCCCCC AGACGAGGT GCCATTTCTG GGGCCGTG

7151 AGGCGCGCGT CGAAGTAGTC TATCTTGCAT CCTTGCAAGT CTAGCGCCTG  
TCCGCGCGCA GCTTCATCAG ATAGAACGTA GGAACGTTCA GATCGCGGAC

7201 CTGCCATGCG CGGGCGGCAA GCGCGCGCTC GTATGGGTTG AGTGGGGAC  
GACGATACG CCGCGCGGT CCGCGCGAG CATACCAAC TCACCCCTG

7251 CCCATGGCAT GGGGTGGGTG AGCGCGGAGG CGTACATGCC GCAAATGTG  
GGGTACCGTA CCCACCCAC TCGCGCTCC GCATGTACGG CGTTTACAGC

7301 TAAACGTAGA GGGGCTCTCT GAGTATTCCA AGATATGTAG GGTAGCATCT  
ATTTGCATCT CCGGAGAGA CTCATAAGGT TCTATACATC CCATCGTAGA

7351 TCCACCGCGG ATGCTGGCGC GCACGTAATC GTATAGTTG TGCGAGGGAG  
AGGTGGCGCC TACGACCGC CGTGCAATTAG CATATCAAGC ACGCTCCCTC

7401 CGAGGAGGTC GGGACGAGG TTGCTACGG CCGGCTGCTC TGCTCGGAAG  
GCTCCTCCAG CCCTGGCTCC AACGATGCCC GCGGACGAG ACGAGCCTTC

7451 ACTATCTGCC TGAAGATGGC ATGTGAGTTG GATGATATGG TTGGACGCTG  
TGATAGACGG ACTTCTACCG TACACTCAAC CTACTATACC AACCTGCGAC

7501 GAAGACGTTG AAGCTGGCGT CTGTGAGACC TACCGCGTCA CGCAGGAAGG  
CTTCTGCAAC TTCGACCGCA GACACTCTGG ATGGCGCAGT GCGTGTCTCC

Figure 26 H

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7551  AGGCGTAGGA GCGCAGC TTGTTGACCA GCTCGGCGGT GACCTGCGG
      TCCGCATCCT CAGCGCGTCG AACAACTGGT CGAGCCGCCA CTGGACGTGC

7601  TCTAGGGCGC AGTAGTCCAG GGTTCCTTG ATGATGTCAT ACTTATCCTG
      AGATCCCGCG TCATCAGGTC CCAAAGGAAC TACTACAGTA TGAATAGGAC

7651  TCCCTTTTTT TTCCACAGCT CGCGGTTGAG GACAACTCT TCGCGGTCTT
      AGGGAAAAAA AAGGTGTCGA GCGCCAACTC CTGTTTGAGA AGCGCCAGAA

7701  TCCAGTACTC TTGGATCGGA AACCCGTCGG CCTCCGAACG GTAAGAGCCT
      AGGTCATGAG AACCTAGCCT TTGGGCAGCC GGAGGCTTGC CATTCTCGGA

7751  AGCATGTAGA ACTGGTTGAC GGCCTGGTAG GCGCAGCATC CCTTTTCTAC
      TCGTACATCT TGACCAACTG CCGGACCATC CGCGTCGTAG GGAAAAGATG

7801  GGGTAGCGCG TATGCCTGCG CGGCCTTCGG GAGCGAGGTG TGGGTGAGCG
      CCCATCGCGC ATACGGACGC GCCGGAAGGC CTCGCTCCAC ACCCACTCGC

7851  CAAAGGTGTC CCTGACCATG ACTTTGAGGT ACTGGTATTT GAAGTCAGTG
      GTTTCACAGG GGACTGGTAC TGAAACTCCA TGACCATAAA CTTCACTCAC

7901  TCGTCGCATC CGCCCTGCTC CCAGAGCAAA AAGTCCGTGC GCTTTTGGGA
      AGCAGCGTAG GCGGGACGAG GGTCTCGTTT TTCAGGCACG CGAAAAACCT

7951  ACGCGGATTT GGCAGGGCGA AGGTGACATC GTTGAAGAGT ATCTTTCCCG
      TGCGCCTAAA CCGTCCCGCT TCCACTGTAG CAACTTCTCA TAGAAAGGGC

8001  CGCGAGGCAT AAAGTTGCGT GTGATGCGGA AGGGTCCCGG CACCTCGGAA
      GCGCTCCGTA TTCAACGCA CACTACGCCT TCCAGGGCC GTGGAGCCTT

8051  CGGTGTGTTA TTACCTGGGC GCGGAGCACG ATCTCGTCAA AGCCGTTGAT
      GCCAACAATT AATGGACCCG CCGCTCGTGC TAGAGCAGTT TCGGCAACTA

8101  GTTGTGGCCC ACAATGTAAA GTTCCAAGAA GCGCGGGATG CCCTTGATGG
      CAACACCGGG TGTTACATTT CAAGGTTCCT CCGGCCCTAC GGGAACTACC

8151  AAGGCAATTT TTTAAGTTCC TCGTAGGTGA GCTCTTCAGG GGAGCTGAGC
      TTCCGTTAAA AAATTCAAGG AGCATCCACT CGAGAAGTCC CCTCGACTCG

8201  CCGTGCTCTG AAAGGGCCCA GTCTGCAAGA TGAGGGTTGG AAGCGACGAA
      GGCACGAGAC TTTCCCGGGT CAGACGTTCT ACTCCCAACC TTCGCTGCTT

8251  TGAGCTCCAC AGGTCACGGG CCATTAGCAT TTGCAGGTGG TCGCGAAAGG
      ACTCGAGGTG TCCAGTGCCC GGTAATCGTA AACGTCCACC AGCGCTTTCC

8301  TCCTAAACTG GCGACCTATG GCCATTTTPT CTGGGGTGAT GCAGTAGAAG
      AGGATTTGAC CGCTGGATAC CGGTAAAAAA GACCCCACTA CGTCATCTTC

8351  GTAAGCGGGT CTTGTTCCCA GCGGTCCCAT CCAAGGTTGG CGGCTAGGTC
      CATTCGCCCC GAACAAGGGT CGCCAGGGTA GGTTCCAAGC GCCGATCCAG

8401  TCGCGCGGCA GTCAGTAGAG GCTCATCTCC GCCGAAC TTCGCTGCTT
      AGCGCGCCGT CAGTGATCTC CGAGTAGAGG CGGCTTGAAG TACTGGTCGT

8451  TGAAGGGCAC GAGCTGCTTC CCAAAGGCCC CCATCCAAGT ATAGGTCTCT
      ACTTCCCGTG CTCGACGAAG GGTTCGCGG GGTAGGTTC TATCCAGAGA

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Figure 26I

8501 ACATCGTAGG TAAAGAG ACGCTCGGTG CGAGGATGCG AGCCGAGG  
 TGTAGCATCC ACTGTTTCTC TGCAGGCCAC GCTCCTACGC TCGGCTAGCC  
 8551 GAAGAACTGG ATCTCCCGCC ACCAATTGGA GGAGTGGCTA TTGATGTGGT  
 CTTCTTGACC TAGAGGGCGG TGGTTAACCT CCTCACCGAT AACTACACCA  
 8601 GAAAGTAGAA GTCCCTGCGA CGGGCCGAAC ACTCGTGCTG GCTTTTGTA  
 CTTTCATCTT CAGGGACGCT GCCCGGCTTG TGAGCACGAC CGAAAACATT  
 8651 AAACGTGCGC AGTACTGGCA GCGGTGCACG GGCTGTACAT CCTGCACGAG  
 TTTGCACGCG TCATGACCGT CGCCACGTGC CCGACATGTA GGACGTGCTC  
 8701 GTTGACCTGA CGACCGCGCA CAAGGAAGCA GAGTGGGAAT TTGAGCCCCT  
 CAACTGGACT GCTGGCGCGT GTTCCTTCGT CTCACCTTA AACTCGGGGA  
 8751 CGCCTGGCGG GTTTGGCTGG TGGTCTTCTA CTTCCGGCTGC TTGTCCTTGA  
 GCGGACCGCC CAAACCGACC ACCAGAAGAT GAAGCCGACG AACAGGAACT  
 8801 CCGTCTGGCT GCTCGAGGGG AGTTACGGTG GATCGGACCA CCACGCCGCG  
 GGCAGACCGA CGAGCTCCCC TCAATGCCAC CTAGCCTGGT GGTGCGGCGC  
 8851 CGAGCCCAA GTCCAGATGT CCGCGCGCGG CGGTCCGAGC TTGATGACAA  
 GCTCGGGTTT CAGGTCTACA GGCAGCGCGC GCCAGCCTCG AACTACTGTT  
 8901 CATCGCGCAG ATGGGAGCTG TCCATGGTCT GGAGCTCCCG CGGCGTCAGG  
 GTAGCGCGTC TACCCTCGAC AGGTACCAGA CCTCGAGGGC GCCGCAGTCC  
 8951 TCAGGCGGGA GCTCCTGCAG GTTTACCTCG CATAGACGGG TCAGGGCGCG  
 AGTCCGCCCT CGAGGACGTC CAAATGGAGC GTATCTGCCC AGTCCCGCGC  
 9001 GGCTAGATCC AGGTGATACC TAATTTCCAG GGGCTGGTTG GTGGCGGCGT  
 CCGATCTAGG TCCACTATGG ATTAAAGTTC CCGGACCAAC CACCGCCGCA  
 9051 CGATGGCTTG CAAGAGGCCG CATCCCCGCG GCGCGACTAC GGTACCGCGC  
 GCTACCGAAC GTTCTCCGGC GTAGGGGCGC GCGCGTGATG CCATGGCGCG  
 9101 GGGCGGCGGT GGGCCCGCGG GGTGTCCTTG GATGATGCAT CTAAAAGCGG  
 CCGCCCGCCA CCCGGCGCCC CCACAGGAAC CTACTACGTA GATTTTCGCC  
 9151 TGACGCGGGC GAGCCCCCGG AGGTAGGGGG GGCTCCGGAC CCGCCGGGAG  
 ACTGCGCCCG CTCGGGGGCC TCCATCCCCC CCGAGGCCTG GGCGGCCCTC  
 9201 AGGGGGCAGG GGCACGTGCG GCGCGCGCGC GGCAGGAGC TGGTGCTGCG  
 TCCCCCGTCC CCGTGCAGCC GCGGCGCGCG CCCGTCTCG ACCACGACGC  
 9251 CGCGTAGGTT GCTGGCGAAC GCGACGACGC GCGGTTGAT CTCCTGAATC  
 GCGCATCAA CGACCGCTTG CGCTGCTGCG CCGCCAATA GAGGACTTAG  
 9301 TGGCGCCTCT GCGTGAAGAC GACGGGCCCG GTGAGCTTGA ACCTGAAAGA  
 ACCGCGGAGA CGCACTTCTG CTGCCCCGGC CACTCGAACT TGGACTTTCT  
 9351 GAGTTGACAA GAATCAATTT CCGTGTCTGT GACGGCGGCC TGGCGCAAAA  
 CTCAAGCTGT CTTAGTTAAA GCCACAGCAA CTGCCGCCGG ACCGCGTTTT  
 9401 TCTCCTGCAC GTCTCCTGAG TTGTCTTGAT AGGCGATCTC GGCCATGAAC  
 AGAGGACGTG CAGAGGACTC AACAGAACTA TCCGCTAGAG CCGGTACTTG

Figure 26 J

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9451  TGCTCGATCT CCTCCTG GAGATCTCCG CGTCCGGCTC GCTCCAAT
      ACGAGCTAGA GAAGGAGGAC CTCTAGAGGC GCAGGCCGAG CGAGGTGCCA

9501  GCGGGCGAGG TCGTTGGAAA TCGGGGCCAT GAGCTGCGAG AAGGCGTTGA
      CCGCCGCTCC AGCAACCTTT ACGCCCGGTA CTCGACGCTC TTCCGCAACT

9551  GGCCTCCCTC GTTCCAGACG CGGCTGTAGA CCACGCCCCC TTCGGCATCG
      CCGGAGGGAG CAAGGTCTGC GCCGACATCT GGTGCGGGGG AAGCCGTAGC

9601  CCGGGCGCGCA TGACCACCTG CGCGAGATTG AGCTCCACGT GCCGGGCGAA
      GCCCGCGCGT ACTGCTGGAC GCGCTCTAAC TCGAGGTGCA CGGCCCGCTT

9651  GACGGCGTAG TTTCCGAGGC GCTGAAAGAG GTAGTTGAGG GTGGTGGCGG
      CTGCCGCATC AAAGCGTCCG CGACTTTCTC CATCAACTCC CACCACCGCC

9701  TGTGTTCTGC CACGAAGAAG TACATAACCC AGCGTCGCAA CGTGGATTGG
      ACACAAGACG GTGCTTCTTC ATGTATTGGG TCGCAGCGTT GCACCTAAGC

9751  TTGATATCCC CCAAGGCCTC AAGGCGCTCC ATGGCCTCGT AGAAGTCCAC
      AACTATAGGG GGTTCGGAG TTCCGCGAGG TACCGGAGCA TCTTCAGGTG

9801  GCGGAAGTTG AAAAAGTGGG AGTTGCGCGC CGACACGGTT AACTCCTCCT
      CCGCTTCAAC TTTTGGACCC TCAACGCGCG GCTGTGCCAA TTGAGGAGGA

9851  CCAGAAGACG GATGAGCTCG GCGACAGTGT CGCGCACCTC GCGCTCAAAG
      GGTCTTCTGC CTACTCGAGC CGCTGTCACA GCGCGTGGAG CGCGAGTTTC

9901  GCTACAGGGG CCTCTTCTTC TTCTTCAATC TCCTCTTCCA TAAGGGCCTC
      CGATGTCCCC GGAGAAGAAG AAGAAGTTAG AGGAGAAGGT ATTCCCGGAG

9951  CCCTTCTTCT TCTTCTGGCG GCGGTGGGGG AGGGGGGACA CGGCGGCGAC
      GGGAAAGAAG AGAAGACCGC CGCCACCCCC TCCCCCTGT GCCGCCGCTG

10001 GACGGCGCAC CGGGAGGCGG TCGACAAAGC GCTCGATCAT CTCCCCGCGG
      CTGCCGCGTG GCCCTCCGCC AGCTGTTTCG CGAGCTAGTA GAGGGGCGCC

10051 CGACGGCGCA TGGTCTCGGT GACGGCGCGG CCGTTCTCGC GGGGGCGCAG
      GCTGCCGCGT ACCAGAGCCA CTGCCGCGCC GGCAAGAGCG CCCCCGCGTC

10101 TTGGAAGACG CCGCCCGTCA TGTCCCGGTT ATGGGTGGGC GGGGGGCTGC
      AACCTTCTGC GGCGGGCAGT ACAGGGCCAA TACCCAACCG CCCCCGACG

10151 CATGCGGCAG GGATACGGCG CTAACGATGC ATCTCAACAA TTGTTGTGTA
      GTACGCCGTC CCTATGCCGC GATTGCTACG TAGAGTTGTT AACAACACAT

10201 GGTACTCCGC CGCCGAGGGA CCTGAGCGAG TCCGCATCGA CCGGATCGGA
      CCATGAGGCG GCGGCTCCCT GGACTCGCTC AGGCGTAGCT GGCCTAGCCT

10251 AAACCTCTCG AGAAAGGCGT CTAACCAGTC ACAGTCGCAA GGTAGGCTGA
      TTTGGAGAGC TCTTCCGCA GATTGGTCAG TGTCAGCGTT CCATCCGACT

10301 GCACCGTGGC GGGCGGCAGC GGGCGGCGGT CGGGGTGTT TCTGGCGGAG
      CGTGGCACCG CCCGCCGTCG CCCGCCGCCA GCCCAACAA AGACCGCCTC

10351 GTGCTGCTGA TGATGTAATT AAAGTAGGCG GTCTTGAGAC GCGGATGGT
      CACGACGACT ACTACATTAA TTTATCCGC CAGAACTCTG CCCCTACCA

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Figure 26 K



10401 CGACAGAAGC AATGTGTCCT TGGGTCCGGC CTGCTGAATG CGCAGGCTT  
 GCTGTCTTCG TGTACAGGA ACCCAGGCCG GACGACTTAC GCGTCCCTCA  
 10451 CGGCCATGCC CCAGGCTTCG TTTTGACATC GGCAGGTC TTTGTAGTAG  
 GCCGGTACGG GGTCCGAAGC AAAACTGTAG CCGCGTCCAG AAACATCATC  
 10501 TCTTGATGA GCCTTTCTAC CGGCACTTCT TCTTCTCCTT CCTCTGTCC  
 AGAACGTACT CGGAAAGATG GCCGTGAAGA AGAAGAGGAA GGAGAACAGG  
 10551 TGCATCTCTT GCATCTATCG CTGCGGCGGC GCGGAGTTT GGCCGTAGGT  
 ACGTAGAGAA CGTAGATAGC GACGCCGCCG CCGCTCAA CCGGCATCCA  
 10601 GCGCCCTCTT TCCTCCCATG CGTGTGACCC CGAAGCCCCT CATCGGCTGA  
 CCGCGGGAGA AGGAGGGTAC GCACACTGGG GCTTCGGGGA GTAGCCGACT  
 10651 AGCAGGGCTA GGTCCGGCAG AACCGCTCG GCTAATATGG CCTGCTGCAC  
 TCGTCCCGAT CCAGCCGCTG TTGCGCGAGC CGATTATACC GGACGACGTG  
 10701 CTGCGTGAGG GTAGACTGGA AGTCATCCAT GTCCACAAAG CCGTGGTATG  
 GACGCACTCC CATCTGACCT TCAGTAGGTA CAGGTGTTTC GCCACCATAC  
 10751 CGCCCGTGTT GATGGTGTA GTGCAGTTGG CCATAACGGA CCAGTTAAGC  
 GCGGGCACAA CTACCACATT CACGTCAACC GGTATTGCCT GGTCAATTGC  
 10801 GTCTGGTGAC CCGGCTGCGA GAGCTCGGTG TACCTGAGAC GCGAGTAAGC  
 CAGACCACTG GGCCGACGCT CTCGAGCCAC ATGGACTCTG CGCTCATTCG  
 10851 CCTCGAGTCA AATACGTAGT CGTTGCAAGT CCGCACCAGG TACTGGTATC  
 GGAGCTCAGT TTATGCATCA GCAACGTTCA GCGTGGTCC ATGACCATAG  
 10901 CCACCAAAAA GTGCGGCGGC GGCTGGCGGT AGAGGGGCCA GCGTAGGGTG  
 GGTGGTTTTT CACGCCGCCG CCGACGCCCA TCTCCCCGGT CGCATCCCAC  
 10951 GCCGGGGCTC CGGGGGCGAG ATCTTCCAAC ATAAGGCGAT GATATCCGTA  
 CGGCCCGGAG GCCCCGCTC TAGAAGGTTG TATTCGCTA CTATAGGCAT  
 11001 GATGTACCTG GACATCCAGG TGATGCCGGC GCGGTGGTG GAGGCGCGCG  
 CTACATGGAC CTGTAGGTCC ACTACGGCCG CCGCCACCAC CTCCGCGCGC  
 11051 GAAAGTCGCG GACGCGGTTC CAGATGTTGC GCAGCGGCAA AAAGTGCTCC  
 CTTTCAGCGC CTGCGCCAAG GTCTACAACG CGTCGCCGTT TTTCACGAGG  
 11101 ATGGTCGGGA CGCTCTGGCC GGTGAGGCGC GCGCAATCGT TGACGCTCTA  
 TACCAGCCCT GCGAGACCGG CCAGTCCGCG CCGGTTAGCA ACTGCGAGAT  
 11151 GACCGTGCAA AAGGAGAGCC TGTAAAGCGG CACTCTTCCG TGGTCTGGTG  
 CTGGCACGTT TTCTCTCGG ACATTGCCCC GTGAGAAGGC ACCAGACCAC  
 11201 GATAAATTCG CAAGGGTATC ATGGCGGACG ACCGGGGTTC GAGCCCCGTA  
 CTATTTAAGC GTTCCCATAG TACCGCCTGC TGGCCCCAAG CTCGGGGCAT  
 11251 TCCGGCCGTC CGCCGTGATC CATGCGGTTA CCGCCCGCGT GTCGAACCCA  
 AGGCCGGCAG GCGGCACTAG GTACGCCAAT GCGGGGCGCA CAGCTTGGGT  
 11301 GGTGTGCGAC GTCAGACAAC GGGGAGTGC TCCTTTTGGC TTCCTTCCAG  
 CCACACGCTG CAGTCTGTTG CCCCCACG AGGAAAACCG AAGGAAGGTC

Figure 26L

11351 GCGCGGCGGC TGGCGCTA GCTTTTTTGG CCACTGGCCG CGCGCACTGT  
 CGCGCCGCGG ACGACGCGAT CGAAAAAACC GGTGACCGGC GCGCGTGGCA  
 11401 AAGCGGTTAG GCTGGAAAGC GAAAGCATT AAGTGGCTCGC TCCCTGTAGC  
 TTCGCCAATC CGACCTTTCG CTTTCGTAAT TCACCGAGCG AGGGACATCG  
 11451 CGGAGGGTTA TTTTCCAAGG GTPGAGTCGC GGGACCCCGC GTTCGAGTCT  
 GCCTCCCAAT AAAAGGTTC CAACTCAGCG CCCTGGGGGC CAAGCTCAGA  
 11501 CGGACCGGCC GGAAGTGGGC GAACGGGGGT TTGCCTCCCC GTCATGCAAG  
 GCCTGCGCGG CCTGACGCGG CTTGCCCCCA AACGGAGGGG CAGTACGTTT  
 11551 ACCCCGCTTG CAAATTCCCT CGGAAACAGG GACGAGCCCC TTTTGTGCTT  
 TGGGGCGAAC GTTTAAGGAG GCCTTTGTCC CTGCTCGGGG AAAAAACGAA  
 11601 TTCCAGATG CATCCGGTGC TGGGCGAGAT GCGCCCCCCT CCTCAGCAGC  
 AAGGTCTAC GTAGGCCACG ACGCCGTCTA CGCGGGGGGA GGAGTCGTCTG  
 11651 GGCAAGAGCA AGAGCAGCGG CAGACATGCA GGGCACCCCT CCCTCCTCCT  
 CCGTCTCTGT TCTCGTGGCC GTCTGTACGT CCCGTGGGAG GGGAGGAGGA  
 11701 ACCGCGTCAG GAGGGGCGAC ATCCGCGGTT GACGCGGCAG CAGATGGTGA  
 TGGCGCAGTC CTCCCCGCTG TAGGCGCCAA CTGCGCCGTC GTCTACCACT  
 11751 TTACGAACCC CCGCGGCGCC GGGCCCCGCA CTACCTGGAC TTGGAGGAGG  
 AATGCTTGGG GCGCGCGCGG CCCGGGCCGT GATGGACCTG AACCTCCTCC  
 11801 GCGAGGGCCT GCGCGGCGTA GGAGCGCCCT CTCCTGAGCG GCACCCAAGG  
 CGCTCCCGGA CCGCGCCGAT CTTGCGGGGA GAGGACTCGC CGTGGGTTC  
 11851 GTGCAGCTGA AGCGTGATAC GCGTGAGGCG TACGTGCCGC GGCAGAACCT  
 CACGTGCACT TCGCACTATG CGCACTCCGC ATGCACGGCG CCGTCTTGGA  
 11901 GTTTCGCGAC CGCGAGGGAG AGGAGCCCGA GGAGATGCGG GATCGAAAGT  
 CAAAGCGCTG GCGCTCCCTC TCCTCGGGCT CCTCTACGCC CTAGCTTTCA  
 11951 TCCACGCAGG GCGCGAGCTG CGGCATGGCC TGAATCGCGA GCGGTGTCTG  
 AGGTGCGTCC CCGGCTCGAC GCGGTACCGG ACTTAGCGCT CGCCAACGAC  
 12001 CGCGAGGAGG ACTTTGAGCC CGACGCGCGA ACCGGGATTA GTCCCGCGCG  
 GCGCTCCTCC TGAAACTCGG GTGCGCGCT TGGCCCTAAT CAGGGCGCGC  
 12051 CGCACACGTG GCGGCCGCGG ACCTGGTAAC CGCATAAGAG CAGACGGTGA  
 GCGTGTGCAC CGCCGGCGGC TGGACCATG GCGTATGCTC GTCTGCCACT  
 12101 ACCAGGAGAT TAACCTTTCAA AAAAGCTTTA ACAACCACGT GCGTACGCTT  
 TGGTCTCTA ATTGAAAGTT TTTTCGAAAT TGTGGGTGCA CGCATGCGAA  
 12151 GTGGCGCGCG AGGAGGTGGC TATAGGACTG ATGCATCTGT GGGACTTTGT  
 CACCGCGCGC TCCTCCACCG ATATCCTGAC TACGTAGACA CCCTGAAACA  
 12201 AAGCGCGCTG GAGCAAAACC CAAATAGCAA GCCGCTCATG GCGCAGCTGT  
 TTCGCGCGAC CTCGTTTTGG GTTTATCGTT CGGCGAGTAC CCGGTCGACA  
 12251 TCCTTATAGT GCAGCACAGC AGGGACAACG AGGCATTAG GGATGCGCTG  
 AGGAATATCA CGTCGTGTCTG TCCCTGTGTC TCCGTAAGTC CCTACGCGAC

Figure 26 M

12301 CTAAACATAG T G C C C G A G G G C C G C T G G C T G C T C G A T T T G A T A A T  
G A T T T G T A T C A T T C G G G C T C C C G G C G A C C G A C G A G C T A A A C T A T T T G T A

12351 C C T G C A G A G C A T A G T G G T G C A G G A G C G C A G C T T G A G C C T G G C T G A C A A G G  
G G A C G T C T C G T A T C A C C A C G T C C T C G C G T C G A A C T C G G A C C G A C T G T T C C

12401 T G G C C G C C A T C A A C T A T T C C A T G C T T A G C C T G G G C A A G T T T T A C G C C C C G  
A C C G G C G G T A G T T G A T A A G G T A C G A A T C G G A C C C G T T C A A A A T G C G G G C G

12451 A A G A T A T A C C A T A C C C C T T A C G T T C C C A T A G A C A A G G A G G T A A A G A T C G A  
T T C T A T A T G G T A T G G G G A A T G C A A G G G T A T C T G T T C C T C C A T T T C T A G C T

12501 G G G G T T C T A C A T G C G C A T G G C G C T G A A G G T G C T T A C C T T G A G C G A C G A C C  
C C C C A A G A T G T A C G C G T A C C G C G A C T T C C A C G A A T G G A A C T C G C T G C T G G

12551 T G G G C G T T T A T C G C A A C G A G C G C A T C C A C A A G C C G T G A G C G T G A G C C G T G A G C C G G  
A C C C G C A A A T A G C G T T G C T C G C G T A G G T G T T C C G G C A C T C G C A C T C G G C C

12601 C G G C G C G A G C T C A G C G A C C G C G A G C T G A T G C A C A G C C T G C A A A G G G C C C T  
G C C G C G C T C G A G T C G C T G G C G C T C G A C T A C G T G T C G G A C G T T T C C C G G G A

12651 G G C T G G C A C G G G C A G C G G C A T A G A G A G G C C G A G T C C T A C T T T G A C G C G G  
C C G A C C G T G C C G T C G C C G C T A T C T C T C C G G C T C A G G A T G A A A C T G C G C C

12701 G C G C T G A C C T G C G C T G G G C C C A A G C C G A C G C G C C C T G G A G G C A G C T G G G  
C C G A C T G G A C G C G A C C C G G G G T T C G G C T G C G C G G A C C T C C G T C G A C C C

12751 G C C G G A C C T G G G C T G G C G G T G G C A C C C G C G C G C G C T G G C A A C G T C G G C G G  
C G G C C T G G A C C G A C C G C C A C C G T G G G C G C G C G A C C G T T G C A G C C G C C

12801 C G T G G A G G A A T A T G A C G A G G A C G A T G A G T A C G A G C C A G A G A C G G C G A G T  
G C A C C T C C T T A T A C T G C T C C T G C T A C T C A T G C T C G G T C T C T G C C G C T C A

12851 A C T A A G C G G T G A T G T T T C T G A T C A G A T G A T G C A A G A C G C A A C G A C C C G G  
T G A T T C G C C A C T A C A A A G A C T A G T C T A C T A C G T T C T G C G T T G C C T G G G C C

12901 C G G T G C G G G C G G C G C T G C A G A G C A G C C G T C C G G C C T T A A C T C C A C G G A C  
G C C A C G C C C G C C G C G A C G T C T C G G T C G G C A G G C C G G A A T T G A G G T G C C T G

12951 G A C T G G C G C C A G G T C A T G G A C C G A T C A T G T C G T G A C T G C G C A A T C C  
C T G A C C G C G G T C C A G T A C C T G G C G T A G T A C A G C G A C T G A C G C G C T T A G G

13001 T G A C G C G T T C C G G C A G C A G C G C A G G C C A C C G G C T C T C C G C A A T T C T G G  
A C T G C G C A A G G C C G T C G T C G G C G C G T C C G G T T G G C C G A G A G G C G T T A A G A C C

13051 A A G C G G T G G T C C C G G C G C G C G C A A A C C C C A C G C A C G A G A A G G T G C T G G C G  
T T C G C C A C C A G G G C C G C G C G C G T T G G G G T G C G T G C T C T T C C A C G A C C G C

13101 A T C G T A A A C G C G T G G C C G A A A C A G G G C C A T C C G G C C C G A C G A G G C C G G  
T A G C A T T T G C G C G A C C G G C T T T G T C C C G G T A G G C C G G G C T G C T C C G G C C

13151 C C T G G T C T A C G A C G C G T G C T T C A G C G C G T G G C T C G T T A C A A C A G C G G C A  
G G A C C A G A T G C T G C G C G A C G A A G T C G C G C A C C G A G C A A T G T T G T C G C C G T

13201 A C G T G C A G A C C A A C C T G G A C C G G C T G G T G G G G A T G T G C G C G A G G C C G T G  
T G C A C G T C T G G T T G A C C T G G C C G A C C A C C T A C A C G C G C T C C G G C A C

Figure 26 N

13251 GCGCAGCGTG ACGCGCA GCAGCAGGCG AACCTGGGCT CCATGGC  
 CGCGTCGCAC TCGCGCGCGT CGTCGTCCCG TTGGACCCGA GGTACCAACG

13301 ACTAAACGCC TTCTTGAGTA CACAGCCCGC CAACGTGCCG CGGGGACAGG  
 TGATTTCGCG AAGGACTCAT GTGTCGGGCG GTTGACCGGC GCCCTGTCC

13351 AGGACTACAC CAACTTTGTG AGCGCACTGC GGCTAATGGT GACTGAGACA  
 TCCTGATGTG GTTGAACAC TCGCGTGACG CCGATTACCA CTGACTCTGT

13401 CCGCAAAGTG AGGTGTACCA GTCTGGGCCA GACTATTTT TCCAGACCAG  
 GCGTTTCAC TCCACATGGT CAGACCCGGT CTGATAAAAA AGGTCTGGTC

13451 TAGACAAGGC CTGCAGACCG TAAACCTGAG CCAGGCTTTC AAAAATTGC  
 ATCTGTTCG GACGTCTGGC ATTTGGACTC GGTCCGAAAG TTTTGAACG

13501 AGGGGCTGTG GGGGGTGCGG GCTCCACAG GCGACCGCG GACCGTGTCT  
 TCCCCGACAC CCCCCACGCC CGAGGGTGTG CGCTGGCGCG CTGGCACAGA

13551 AGCTTGCTGA CGCCAACTC GCGCTGTG CTGCTGCTAA TAGCGCCCTT  
 TCGAACGACT GCGGGTTGAG CGCGGACAAC GACGACGATT ATCGCGGGAA

13601 CACGGACAGT GGCAGCGTGT CCCGGGACAC ATACCTAGGT CACTTGCTGA  
 GTGCCTGTCA CCGTCGCACA GGGCCCTGTG TATGGATCCA GTGAACGACT

13651 CACTGTACCG CGAGGCCATA GGTCAGGCGC ATGTGGACGA GCATACTTTC  
 GTGACATGGC GCTCCGGTAT CCAGTCCGCG TACACCTGCT CGTATGAAAG

13701 CAGGAGATTA CAAGTGTCAG CCGCGCGCTG GGGCAGGAGG ACACGGGCAG  
 GTCCTCTAAT GTTCACAGTC GGCAGCGGAC CCCGTCTCC TGTGCCCCGTC

13751 CCTGGAGGCA ACCCTAACT ACCTGCTGAC CAACCGGCGG CAGAAGATCC  
 GGACCTCCGT TGGGATTTGA TGGACGACTG GTTGGCCGCC GTCTTCTAGG

13801 CCTCGTTGCA CAGTTTAAAC AGCGAGGAGG AGCGCATTTT GCGCTACGTG  
 GGAGCAACGT GTCAAATTTG TCGCTCCTCC TCGCGTAAAA CGCGATGCAC

13851 CAGCAGAGCG TGAGCCTTAA CCTGATGCGC GACGGGGTAA CGCCAGCGT  
 GTCGTCTCGC ACTCGGAATT GGACTACGCG CTGCCCCATT GCGGTCGCA

13901 GCGCGTGGAC ATGACCGCGC GCAACATGGA ACCGGGCATG TATGCCCTCAA  
 CCGCGACCTG TACTGGCGCG CGTTGTACCT TGGCCCGTAC ATACGGAGTT

13951 ACCGGCCGTT TATCAACCGC CTAATGGACT ACTTGCACTG CGCGGCCGCC  
 TGGCCGGCAA ATAGTTGGCG GATTACCTGA TGAACGTAGC GCGCCGGCGG

14001 GTGAACCCCG AGTATTTTAC CAATGCCATC TTGAACCCGC ACTGGCTACC  
 CACTTGGGGC TCATAAAGTG GTTACGGTAG AACTTGGGCG TGACCGATGG

14051 GCCCCCTGGT TTCTACACCG GGGGATTCGA GGTGCCCCAG GGTAAACGATG  
 CGGGGGACCA AAGATGTGGC CCCCTAAGCT CCACGGGCTC CCAATTGCTAC

14101 GATTCTCTCTG GGCAGACATA GACGACAGCG TGTTTTCCCC GCAACCGCAG  
 CTAAGGAGAC CCTGCTGTAT CTGCTGTGCG ACAAAGGGG CGTTGGCGTC

14151 ACCCTGCTAG AGTTGCAACA GCGCGAGCAG GCAGAGGCGG CGCTCGGAAA  
 TGGGACGATC TCAACGTTGT CGCGCTCGTC CGTCTCCGCC GCGACGCTTT

Figure 260

14201 GGAAAGCTTC CAGAGGCCAA GCAGCTTGTC CGATCTAGGC GCTGCGGCTC  
 CCTTTTCGAAG GTCGCGGT CGTCGAACAG GCTAGATCCG GCACGCGG

14251 CGCGGTCAGA TGCTAGTAGC CCATTTCCAA GCTTGATAGG GTCTCTTACC  
 GCGCCAGTCT ACGATCATCG GGTAAAGGTT CGAACTATCC CAGAGAATGG

14301 AGCACTCGCA CCACCCGCCC GCGCCTGCTG GCGGAGGAGG AGTACCTAAA  
 TCGTGAGCGT GGTGGGCGGG CCGGACGAC CCGCTCCTCC TCATGGATTT

14351 CAACTCGCTG CTGCAGCCGC AGCGCGAAAA AAACCTGCCT CCGGCATTTC  
 GTTGAGCGAC GACGTCGGCG TCGCGCTTTT TTTGGACGGA GGCCGTAAAG

14401 CCAACAACGG GATAGAGAGC CTAGTGGACA AGATGAGTAG ATGGAAGACG  
 GGTGTTGCC CTATCTCTCG GATCACCTGT TCTACTCATC TACCTTCTGC

14451 TACGCGCAGG AGCACAGGGA CGTGCCAGGC CCGCGCCCGC CCACCCGTCG  
 ATGCGCGTCC TCGTGTCCTT GCACGGTCCG GCGCGGGGCG GGTGGGCAGC

14501 TCAAAGGCAC GACCGTCAGC GGGGTCTGGT GTGGGAGGAC GATGACTCGG  
 AGTTTCCGTG CTGSCAGTCG CCCCAGACCA CACCCTCCTG CTACTGAGCC

14551 CAGACGACAG CAGCGTCCTG GATTTGGGAG GGAGTGGCAA CCCGTTTGCG  
 GTCTGCTGTC GTCGAGGAC CTAACCCCTC CCTCACCCTT GGGCAAACGC

14601 CACCTTCGCC CCAGGCTGGG GAGAATGTTT TAAAAAATAA AAAAGCATGA  
 GTGGAAGCGG GGTCCGACCC CTCTTACAAA ATTTTTTTTT TTTTCGTACT

14651 TGCAAAATAA AAAACTCACC AAGGCCATGG CACCGAGCGT TGGTTTTCTT  
 ACGTTTTATT TTTGAGTGG TTCCGGTACC GTGGCTCGCA ACCAAAAGAA

14701 GTATTCCCCT TAGTATGCGG CGCGCGGCGA TGTATGAGGA AGGTCTCTCT  
 CATAAGGGGA ATCATACGCC GCGCGCCGCT ACATACTCTT TCCAGGAGGA

14751 CCCTCTTACG AGAGTGTGGT GAGCGCGGCG CCAGTGGCGG CGGCGCTGGG  
 GGGAGGATGC TCTCACACCA CTCGCGCCGC GGTACCCGCC GCGCGACCC

14801 TTCTCCCTTC GATGCTCCCC TGGACCCGCC GTTTGTGCCT CCGCGGTACC  
 AAGAGGGGAA CTACGAGGGG ACCTGGGCGG CAAACACGGA GCGGCCATGG

14851 TGCGGCCTAC CGGGGGGAGA AACAGCATCC GTTACTCTGA GTTGGCACCC  
 ACGCCGGATG GCCCCCTCT TTGTCTAGG CAATGAGACT CAACCGTGGG

14901 CTATTTCGACA CCACCCGTGT GTACCTGGTG GACAACAAGT CAACGGATGT  
 GATAAGCTGT GGTGGGCACA CATGGACCAC CTGTTGTTCA GTTGCCTACA

14951 GGCATCCCTG AACTACCAGA ACGACCACAG CAACTTTCTG ACCACGGTCA  
 CCGTAGGGAC TTGATGGTCT TGCTGGTGTC GTTGAAAGAC TGGTGCCAGT

15001 TTCAAACAA TGAATACAGC CCGGGGAGG CAAGCACACA GACCATCAAT  
 AAGTTTTGTT ACTGATGTCG GGCCCCCTCC GTTCGTGTGT CTGGTAGTTA

15051 CTGACGACC GGTGCACTG GGGCGGCGAC CTGAAAACCA TCCTGCATAC  
 GAACTGCTGG CCAGCGTGAC CCGCGCGCTG GACTTTTGGT AGGACGTATG

15101 CAACATGCCA AATGTGAACG AGTTTCATGT TACCAATAAG TTTAAGGCGC  
 GTGTACGGT TTACACTTGC TCAAGTACAA ATGGTTATTC AAATTCGCG

Figure 26 P

15151 GGGTGATGGT CCGCTTG CCTACTAAGG ACAATCAGGT GGAGCTA  
 CCCACTACCA CAGCGCGAAC GGATGATTCC TGTTAGTCCA CCTCGACTTT  
 15201 TACGAGTGGG TGGAGTTCAC GCTGCCCCGAG GGCAACTACT CCGAGACCAT  
 ATGCTCACCC ACCTCAAGTG CGACGGGCTC CCGTTGATGA GGCTCTGGTA  
 15251 GACCATAGAC CTTATGAACA ACGCGATCGT GGAGCACTAC TTGAAAGTGG  
 CTGGTATCTG GAATACTTGT TCGGCTAGCA CCTCGTGATG AACTTTCACC  
 15301 GCAGACAGAA CGGGGTTCG GAAAGCGACA TCGGGGTAAA GTTTGACACC  
 CGTCTGTCTT GCCCAAGAC CTTTCGCTGT AGCCCCATT CAAACTGTGG  
 15351 CGCAACTTCA GACTGGGGTT TGACCCCGTC ACTGGTCTTG TCATGCCTGG  
 CGGTTGAAGT CTGACCCCAA ACTGGGCGAG TGACCAGAAC AGTACGGACC  
 15401 GGTATATACA AACGAAGCCT TCCATCCAGA CATCATTTTG CTGCCAGGAT  
 CCATATATGT TTGCTTCGGA AGGTAGTCT GTAGTAAAC GACGGTCCTA  
 15451 GCGGGGTGGA CTTCAACCCAC AGCCGCCTGA GCAACTTGT GGGCATCCGC  
 CGCCCCACCT GAAGTGGGTG TCGGCGGACT CGTTGAACAA CCCGTAGGCG  
 15501 AAGCGGCAAC CCTTCAGGA GGGCTTTAGG ATCACCTACG ATGATCTGGA  
 TTCGCGCTTG GGAAGGTCTT CCCGAAATCC TAGTGGAATG TACTAGACCT  
 15551 GGGTGGAAC ATTCCCGCAC TGTGGATGT GGACGCCTAC CAGGCGAGCT  
 CCCACCATTG TAAGGGCGTG ACAACCTACA CCTGCGGATG GTCCGCTCGA  
 15601 TGAAAGATGA CACCGAACAG GCGGGGGTG GCGCAGGCG CAGCAACAGC  
 ACTTCTACT GTGGCTGTC CCGCCCCAC CGCGTCCGCC GTCGTGTGTC  
 15651 AGTGGCAGCG GCGCGGAAGA GAACTCCAAC GCGGCAGCG CGGCAATGCA  
 TCACCCTGCG CGCGCCTTCT CTTGAGGTG CGCCGTCCGC GCCGTACTGT  
 15701 GCCGTGGAG GACATGAACG ATCATGCCAT TCGCGCGAC ACCTTTGCCA  
 CGGCCACCTC CTGTACTTGC TAGTACGGTA AGCGCCGCTG TGGAAACGGT  
 15751 CACGGGCTGA GGAGAAGCG GCTGAGGCG AAGCAGCGGC CGAAGCTGCC  
 GTGCCGACT CCTCTTCGCG GACTCCGCG TCGTCCGCC GCTTCGACGG  
 15801 GCCCCGCTG CGCAACCGA GTCGAGAAG CCTCAGAAGA AACCAGGTAT  
 CGGGGCGGAC CGGTTGGGCT CCAGCTCTTC GGAGTCTTCT TTGGCCACTA  
 15851 CAAACCCCTG ACAGAGGACA GCAAGAAACG CAGTTACAAC CTAATAAGCA  
 GTTTGGGGAC TGTCTCTGT CGTCTTTGC GTCAATGTTG GATTATTCTG  
 15901 ATGACAGCAC CTTACCCAG TACCGCAGCT GGTACCTTGC ATACAACCTAC  
 TACTGTCTG GAAGTGGGTC ATGGCGTCGA CCATGGAACG TATGTTGATG  
 15951 GGCAGCCCTC AGACCGGAAT CCGCTCATGG ACCCTGCTTT GCACTCCTGA  
 CCGCTGGGAG TCTGGCCTTA GCGAGTACC TGGGACGAAA CGTGAGGACT  
 16001 CGTAACCTGC GGCTCGGAGC AGGTCTACTG GTCGTTGCCA GACATGATGC  
 GCATTGGACG CCGAGCCTCG TCCAGATGAC CAGCAACGGT CTGTACTACG  
 16051 AAGACCCCGT GACCTTCCGC TCCACGCGCC AGATCAGCAA CTTTCCGGTG  
 TTCTGGGGCA CTGGAAGCGG AGGTGCGCGG TCTAGTCGTT GAAAGGCCAC

Figure 26 Q

16101 GTGGGCGCCG A TGGTTGCC CGTGCACTCC AAGAGCTTCT ACAACG TCA  
 CACCCGCGGC T TACAACGG GCACGTGAGG TTCTCGAAGA TGTTGC TST  
 16151 GGCCGTCTAC TCCCAACTCA TCCGCCAGTT TACCTCTCTG ACCCACGTGT  
 CCGGCAGATG AGGGTTGAGT AGGCGGTCAA ATGGAGAGAC TGGGTGCACA  
 16201 TCAATCGCTT TCCCGAGAAC CAGATTTTGG CGCGCCCGCC AGCCCCACC  
 AGTTAGCGAA AGGGCTCTTG GTCTAAAACC GCGCGGGCGG TCGGGGGTGG  
 16251 ATCACCACCG TCAGTGA AAA CGTTCTTGCT CTCACAGATC ACGGGACGCT  
 TAGTGGTGGC AGTCAC TTTT GCAAGGACGA GAGTGTCTAG TGCCCTGCGA  
 16301 ACCGCTGCGC AACAGCATCG GAGGAGTCCA GCGAGTGACC ATTACTGACG  
 TGGCGACGCG TTGTCTG TAGC CTCCTCAGGT CGCTCACTGG TAATGACTGC  
 16351 CCAGACGCCG CACCTGCCCC TACGTTTACA AGGCCCTGGG CATAGTCTCG  
 GGTCTGCGGC GTGGACGGGG ATGCAAATGT TCCGGGACCC GTATCAGAGC  
 16401 CCGCGCGTCC TATCGAGCCG CACTTTTGA GCAAGCATGT CCATCCTTAT  
 GCGCGCAGG ATAGCTCGGC GTGAAAACT CGTTCGTACA GGTAGGAATA  
 16451 ATCGCCACAG AATAACACAG GCTGGGGCCT GCGCTTCCCA AGCAAGATGT  
 TAGCGGGTCG TTATTGTGTC CGACCCCGGA CGCGAAGGT TCGTTCTACA  
 16501 TTGGCGGGGC CAAGAAGCGC TCCGACCAAC ACCCAGTGCG CGTGCGCGGG  
 AACCGCCCCG GTTCTTCGCG AGGCTGGTTG TGGGTACGC GCACGCGCCC  
 16551 CACTACCGCG CGCCCTGGGG CGCGCACAAA CGCGGCCGCA CTGGGCGCAC  
 GTGATGGCGC GCGGGACCCC GCGCGTGT T GCGCCGCGT GACCCGCGTG  
 16601 CACCGTCGAT GACGCCATCG ACGCGGTGGT GGAGGAGGCG CGCAACTACA  
 GTGGCAGCTA CTGCGGTAGC TGCGCCACCA CCTCCTCCGC GCGTTGATGT  
 16651 CGCCACGCC GCCACAGTG TCCACAGTGG ACGCGGCCAT TCAGACCGTG  
 GCGGGTGC GG TGGTGCAC AGGTGTCACC TGCGCCGTA AGTCTGGCAC  
 16701 GTGCGCGGAG CCCGGCGCTA TGCTAAAATG AAGAGACGGC GGAGGCGCGT  
 CACGCGCCTC GGGCCGCGAT ACGATTTTAC TTCTCTGCCG CCTCCGCGCA  
 16751 AGCACGTGCG CACCGCCGCC GACCCGGCAC TGCCGCCCAA CGCGCGGCGG  
 TCGTGCAGCG GTGGCGGCGG CTGGGCCGTG ACGCGGGGT SCGCGCCGCC  
 16801 CGGCCCTGCT TAACCGCGCA CGTCGCACCG GCCGACGGC GGCCATGCGG  
 GCCGGGACGA ATTGGCGCGT GCAGCGTGGC CGGCTGCCCG CCGGTACGCC  
 16851 GCGCTCGAA GGCTGGCCGC GGGTATTGTC ACTGTGCCCC CCAGGTCCAG  
 CCGCGAGCTT CCGACCGGCG CCCATAACAG TGACACGGGG GTTCCAGGTC  
 16901 GCGACGAGCG GCCGCCGAG CAGCCGCGGC CATTAGTGCT ATGACTCAGG  
 CGCTGCTCGC CGGCGGCGTC GTCGGCGCCG GTAATCACGA TACTGAGTCC  
 16951 GTCGAGGGG CAACGTGTAT TGGGTGCGCG ACTCGGTTAG CGGCCTGCGC  
 CAGCGTCCCC GTTGACATA ACCCACGCGC TGAGCCAATC GCCGGACGCG  
 17001 GTCCCCGTGC GCACCCGCC CCCGCGCAAC TAGATTGCAA GAAAAACTA  
 CACGGGCACG CGTGGGCGGG GGGCGCGTTG ATCTAACGTT CTTTTTGAT

Figure 26 R

17051 CTTAGACTCG TTTGTTGTA TGTATCCAGC GCGGGCGGCG CCGAACCTG  
 GAATCTGAGC ATGACAACAT ACATAGGTCG CCGCCGCCGC GCGTTGCTTC

17101 CTATGTCCAA GCGCAAAATC AAAGAAGAGA TGCTCCAGGT CATCGCGCCG  
 GATACAGGTT CCGGTTTTAG TTTCTTCTCT ACGAGGTCCA GTAGCGCGGC

17151 GAGATCTATG GCGCCCCGAA GAAGGAAGAG CAGGATTACA AGCCCCGAAA  
 CTCTAGATAC CGGGGGGCTT CTTCTTCTCT GTCCTAATGT TCGGGGCTTT

17201 GCTAAAGCGG GTCAAAAAGA AAAAGAAAGA TGATGATGAT GAACTTGACG  
 CGATTTCCGC CAGTTTTTCT TTTCTTTCT ACTACTACTA CTGGAACGTC

17251 ACGAGGTGGA ACTGCTGCAC GCTACCGCGC CCAGGCGACG GGTACAGTGG  
 TGCTCCACCT TGACGACGTG CGATGGCGCG GGTCCGCTGC CCATGTCACC

17301 AAAGGTGCGC GCGTAAACG TGTTTTCCGA CCCGGCACCA CCGTAGTCTT  
 TTTCCAGCTG CGCATTTTGC ACAAACGCT GGGCCGTGGT GGCATCAGAA

17351 TACGCCCCGT GAGCGCTCCA CCCGCACCTA CAAGCGCGTG TATGATGAGG  
 ATCGGGGCCA CTCGCGAGGT GGGCGTGGAT GTTCGCGCAC ATACTACTCC

17401 TGTACGGCGA CGAGGACCTG CTTGAGCAGG CCAACGAGCG CCTCGGGGAG  
 ACATGCCGCT GCTCCTGGAC GAACTCGTCC GGTGCTCGC GGAGCCCCTC

17451 TTTGCCCTACG GAAAGCGGCA TAAGGACATG CTGGCGTTGC CGCTGGACGA  
 AAACGGATGC CTTTCGCCGT ATTCTGTAC GACCGCAACG GCGACCTGCT

17501 GGGCAACCCA ACACCTAGCC TAAAGCCCGT AACACTGCAG CAGGTGCTGC  
 CCCGTTGGGT TGTGGATCGG ATTTCCGGCA TTGTGACGTC GTCCACGACG

17551 CCGCGCTTGC ACCGTCCGAA GAAAAGCGCG GCCTAAAGCG CGAGTCTGGT  
 GGGCGGAACG TGGCAGGCTT CTTTTCCGCG CGGATTTCCG GCTCAGACCA

17601 GACTTGGCAC CCACCGTGCA GCTGATGGTA CCCAAGCGCC AGCGACTGGA  
 CTGAACCGTG GGTGGCACGT CGACTACCAT GGGTTCGCGG TCGCTGACCT

17651 AGATGTCTTG GAAAAATGA CCGTGAAC CTTGGCTGGAG CCGAGGTCC  
 TCTACAGAAC CTTTTTACT GGCACCTTGG ACCCGACCTC GGGCTCCAGG

17701 GCGTGGCGCC AATCAAGCAG GTGGCGCCGG GACTGGGCGT GCAGACCGTG  
 CGCACGCCGG TTAGTTCGTC CACCGCGGCC CTGACCCGCA CGTCTGGCAC

17751 GACGTTTCTG TACCCACTAC CAGTAGCACC AGTATTGCCA CCGCCACAGA  
 CTGCAAGTCT ATGGGTGATG GTCATCGTGG TCATAACGGT GCGGGTGTCT

17801 GGGCATGGAG ACACAAACGT CCGCGTTTGC CTCAGCGGTG GCGGATGCCG  
 CCCGTACCTC TGTGTTTCCA GGGGCCAACG GAGTCGCCAC CGCCTACGGC

17851 CCGTCCAGGC GGTGCGTGGC GCGCGCTCCA AGACCTCTAC GGAGGTGCAA  
 GCCACGTCCG CCAGCGACGC CGGCGCAGGT TCTGGAGATG CCTCCACGTT

17901 ACGGACCCGT GGATGTTTCG CTTTTTCAGC CCGCGCGGCC CGCGCCGTTT  
 TGCTTGGGCA CCTACAAAGC GCAAAGTCGG GGGGCCGCGG GCGCGGCAAG

17951 GAGGAAGTAC GCGCGCGCCA GCGCGCTACT GCGCGAATAT GCCCTACATC  
 CTCTTTCATG CCGCGGCGGT CCGCGCATGA CCGGCTTATA GGGGATGTAG

Figure 265



18001 CTTCCATTGC GCCTACCCCC GGCTATCGTG GCTACACCTA CCGGCGCGTGA  
 GAAGGTAACG CATTGGGGG CCGATAGCAC CGATGTGGAT GCGGGGCTT  
 18051 AGACGAGCAA CTACCCGACG CCGAACCACC ACTGGAACCC GCCGCCGCCG  
 TCTGCTCGTT GATGGGCTGC GGCTTGGTGG TGACCTTGGG CCGCGCGCGC  
 18101 TCGCCGTGCG CAGCCCCGTGC TGGCCCCGAT TTCCGTGCGC AGGGTGGCTC  
 AGCGGCAGCG GTCGGGCACG ACCGGGGCTA AAGGCACGCG TCCCACCGAG  
 18151 GCGAAGGAGG CAGGACCCTG GTGCTGCCAA CAGCGCGCTA CCACCCACG  
 CGCTTCCTCC GTCTGGGAC CACGACGGTT GTCGCGCGAT GGTGGGGTGG  
 18201 ATCGTTTAAA AGCCGGTCTT TGTGGTTCTT GCAGATATGG CCCTCACCTG  
 TAGCAAATTT TCGGCCAGAA ACACCAAGAA CGTCTATACC GGGAGTGGAC  
 18251 CCGCCTCCGT TTCCCGGTGC CCGGATTCCG AGGAAGAATG CACCGTAGGA  
 GCGCGAGGCA AAGGGCCACG GCCCTAAGGC TCCTTCTTAC GTGGCATCCT  
 18301 GGGGCATGGC CGGCCACGGC CTGACGGGCG GCATGCGTCG TCGGCACCAC  
 CCCCCTACCG GCCGGTGCCG GACTGCCCCG CGTACGCAGC ACGCGTGGTG  
 18351 CGCGGGCGGC GCGCGTCGCA CCGTCGCATG CGCGGCGGTA TCCTGCCCTT  
 GCCGCCGCCG CCGCGAGCGT GGCAGCGTAC GCGCCGCCAT AGGACGGGGA  
 18401 CCTTATTCCA CTGATCGCCG CGGCGATTGG CGCCGTGCCC GGAATTGCAT  
 GGAATAAGGT GACTAGCGGC GCCGCTAACC GCGGCACGGG CCTTAACGTA  
 18451 CCGTGGCCTT GCAGGCGCAG AGACACTGAT TAAAAACAAG TTGCATGTGG  
 GGCACCGGAA CGTCCGCGTC TCTGTGACTA ATTTTGTTC AACGTACACC  
 18501 AAAAATCAAA ATAAAAAGTC TGGACTCTCA CGCTCGCTTG GTCCTGTAAC  
 TTTTtagTTT TATTTTTCAG ACCTGAGAGT GCGAGCGAAC CAGGACATTG  
 18551 TATTTTGTAG AATGGAAGAC ATCAACTTTG CGTCTCTGGC CCCGCGACAC  
 ATAAACATC TTACCTTCTG TAGTTGAAAC GCAGAGACCG GGGCGCTGTG  
 18601 GGCTCGCGCC CGTTCATGGG AAAC TGSCAA GATATCGGCA CCAGCAATAT  
 CCGAGCGCGG GCAAGTACCC TTTGACCGTT CTATAGCCGT GGTGCTTATA  
 18651 GAGCGGTGGC GCCTTCAGCT GGGGCTCGCT GTGGAGCGGC ATTAAAAATT  
 CTCGCCACCG CGGAAGTCGA CCCCAGCGCA CACCTCGCCG TAATTTTAA  
 18701 TCGGTTCCAC CGTTAAGAAC TATGGCAGGA AGGCCTGGAA CAGCAGCACA  
 AGCCAAGGTG GCAATTCTTG ATACCGTCGT TCCGGACCTT GTCGTCTGTG  
 18751 GGCCAGATGC TGAGGGATAA GTTGAAGAG CAAAATTTC AACAAAAGGT  
 CCGGTCTACG ACTCCCTATT CAACTTTCTC GTTTTAAAGG TTGTTTCCA  
 18801 GGTAGATGGC CTGGCCTCTG GCATTAGCGG GGTGGTGGAC CTGGCCAACC  
 CCATCTACCG GACCGGAGAC CGTAATCGCC CCACCACCTG GACCGGTGG  
 18851 AGGCAGTGCA AAATAAGATT AACAGTAAGC TTGATCCCCG CCCTCCCGTA  
 TCCGTACAGT TTTATTCTAA TTGTCATTG AACTAGGGGC GGGAGGGCAT  
 18901 GAGGAGCCTC CACCGGCCGT GGAGACAGTG TCTCCAGAGG GGCGTGGCGA  
 CTCTCTGGAG GTGGCCGGCA CCTCTGTAC AGAGGTCTCC CCGCACCGCT

Figure 26T

18951 AAAGCGTCCG CCGACA GGAAGAAAC TCTGGTGACG CAAATACG  
 TTTCGCAGGC GCGGGCTGT CCCTTCTTTG AGACCACTGC GTTATC

19001 AGCCTCCCTC GTACGAGGAG GCACTAAAGC AAGGCCTGCC CACCACCCGT  
 TCGGAGGGAG CATGCTCCTC CGTGATTTCG TTCCGGACGG GTGGTGGGCA

19051 CCCATCGCGC CCATGGCTAC CGGAGTGCTG GGCCAGCACA CACCCGTAAC  
 GGGTAGCGCG GGTACCGATG GCCTCACGAC CCGGTCGTGT GTGGGCATTG

19101 GCTGGACCTG CCTCCCCCG CCGACACCCA GCAGAAACCT GTGCTGCCAG  
 CGACCTGGAC GGAGGGGGGC GGCTGTGGT CGTCTTTGGA CACGACGGTC

19151 GCCCGACCGC CGTTGTTGTA ACCCGTCCTA GCCGCGCGTC CCTGCGCCGC  
 CGGGCTGGCG GCAACAACAT TGGGCAGGAT CGGCGCGCAG GGACGCGGCG

19201 GCCGCCACGC GTCCGCGATC GTTGCGGCCC GTAGCCAGTG GCAACTGGCA  
 CGGCGGTGCG CAGGCGCTAG CAACGCCGGG CATCGGTCAC CGTTGACCGT

19251 AAGCACACTG AACAGCATCG TGGGTCTGGG GGTGCAATCC CTGAAGCGCC  
 TTCGTGTGAC TTGTCTAGC ACCCAGACCC CCACGTTAGG GACTTCGCGG

19301 GACGATGCTT CTGATAGCTA ACGTGTCGTA TGTGTGTCAT GTATGCGTCC  
 CTGCTACGAA GACTATCGAT TGCACAGCAT ACACACAGTA CATACGCAGG

19351 ATGTCGCCGC CAGAGGAGCT GCTGAGCCGC CGCGCGCCCG CTTTCCAAGA  
 TACAGCGGCG GTCTCCTCGA CGACTCGGCG CGCGCGGGCG GAAAGGTTCT

19401 TGGTACCCC TTCGATGATG CCGCAGTGGT CTTACATGCA CATCTCGGGC  
 ACCGATGGGG AAGCTACTAC GGCCTCACCA GAATGTACGT GTAGAGCCCC

19451 CAGGACGCCT CGGAGTACCT GAGCCCCGGG CTGGTGCACT TTGCCCCGCG  
 GTCTCGCGGA GCCTCATGGA CTCGGGGCCC GACCACGTCA AACGGGCGCG

19501 CACCAGAGACG TACTTCAGCC TGAATACAA GTTTAGAAAC CCCACGGTGG  
 GTGGCTCTGC ATGAAGTCGG ACTTATTGTT CAAATCTTTG GGGTGCCACC

19551 CGCCTACGCA CGACGTGACC ACAGACCGGT CCCAGCGTTT GACGCTGCGG  
 GCGGATGCGT GCTGCACTGG TGTCTGGCCA GGGTCGCAA CTGCGACGCC

19601 TTCATCCCTG TGGACCGTGA GGATACTGCG TACTCGTACA AGGCGCGGTT  
 AAGTAGGGAC ACCTGGCACT CCTATGACGC ATGAGCATGT TCCGCGCCAA

19651 CACCCTAGCT GTGGGTGATA ACCGTGTGCT GGACATGGCT TCCACGTACT  
 GTGGGATCGA CACCCACTAT TGGCACACGA CCTGTACCGA AGGTGCATGA

19701 TTGACATCCG CGGCGTGCTG GACAGGGGCC CTACTTTTAA GCCCTACTCT  
 AACTGTAGGC GCCGCACGAC CTGTCCCCGG GATGAAAATT CGGGATGAGA

19751 GGGACTGCCT ACAACGCCCT GGCTCCCAAG GGTGCCCCAA ATCCTTGCGA  
 CCGTGACGGA TGTTCGGGA CCGAGGGTTC CCACGGGGTT TAGGAACGCT

19801 ATGGGATGAA GCTGCTACTG CTCTTGAAAT AAACCTAGAA GAAGAGGACG  
 TACCTTACTT CGACGATGAC GAGAACTTTA TTTGGATCTT CTCTCCTGC

19851 ATGACAACGA AGACGAAGTA GACGAGCAAG CTGAGCAGCA AAAAATCAC  
 TACTGTTGCT TCTGCTTCAT CTGCTCGTTC GACTCGTCGT TTTTGTAGTG

Figure 26 u

19901 GTATTTGGGC A GCCTTA TTCTGGTATA AATATTACAA AGGAGG T  
 CATAAACCCG TCCGCGGAAT AAGACCATAT TTATAATGTT TCCTCCCATTA  
 19951 TCAAATAGGT GTCGAAGGTC AAACACCTAA ATATGCCGAT AAAACATTTC  
 AGTTTATCCA CAGCTTCCAG TTTGTGGATT TATACGGCTA TTTTGTAAAG  
 20001 AACCTGAACC TCAAATAGGA GAATCTCAGT GGTACGAAAC AGAAATTAAT  
 TTGGACTTGG AGTTTATCCT CTTAGAGTCA CCATGCTTGG TCTTTAATTA  
 20051 CATGCAGCTG GGAGAGTCCT AAAAAAGACT ACCCCAATGA AACCATGTTA  
 GTACGTCGAC CCTCTCAGGA TTTTTCCTGA TGGGGTACT TTGGTACAAT  
 20101 CGGTTTCATAT GCAAAACCCA CAAATGAAAA TGGAGGGCAA GGCATTCTTG  
 GCCAAGTATA CGTTTGGGT GTTACTTTT ACCTCCCGTT CCGTAAGAAC  
 20151 TAAAGCAACA AAATGGAAAG CTAGAAAGTC AAGTGGAAAT GCAATTTTTC  
 ATTTCGTTGT TTTACCTTTC GATCTTTCAG TTCACCTTTA CGTTAAAAAG  
 20201 TCAACTACTG AGGCAGCCGC AGGCAATGGT GATAACTTGA CTCCTAAAGT  
 AGTTGATGAC TCCGTCGGCG TCCGTTACCA CTATTGAACT GAGGATTTC  
 20251 GGTATTGTAC AGTGAAGATG TAGATATAGA AACCCAGAC ACTCATATTT  
 CCATAACATG TCACTTCTAC ATCTATATCT TTGGGGTCTG TGAGTATAAA  
 20301 CTTACATGCC CACTATTAAG GAAGGTAAGT CACGAGAACT AATGGGCCAA  
 GAATGTACGG GTGATAATTC CTTCCATTGA GTGCTCTGA TTACCCGGTT  
 20351 CAATCTATGC CCAACAGGCC TAATTACATT GCTTTTAGGG ACAATTTTAT  
 GTTAGATACG GGTGTGCCG ATTAATGTAA CGAAATCCC TGTTAAAAATA  
 20401 TGGTCTAATG TATTACAACA GCACGGGTAA TATGGGTGTT CTGGCGGGCC  
 ACCAGATTAC ATAATGTTGT CGTGCCCAT TATACCCACAA GACCGCCCGG  
 20451 AAGCATCGCA GTTGAATGCT GTTGTAGATT TGCAAGACAG AAACACAGAG  
 TTCGTAGCGT CAACTTACGA CAACATCTAA ACGTTCGTG TTTGTGTCTC  
 20501 CTTTCATACC AGCTTTTGCT TGATTCCATT GGTGATAGAA CCAGGTACTT  
 GAAAGTATGG TCGAAAACGA ACTAAGGTAA CCACTATCTT GGTCCATGAA  
 20551 TTCTATGTGG AATCAGGCTG TTGACAGCTA TGATCCAGAT GTTAGAATTA  
 AAGATACACC TTAGTCCGAC AACTGTGAT ACTAGGTCTA CAATCTTAAT  
 20601 TTGAAAATCA TGGAAGTGA GATGAAGTTC CAAATTACTG CTTTCCACTG  
 AACTTTTAGT ACCTTGACTT CTACTTGAAG GTTTAATGAC GAAAGGTGAC  
 20651 GGAGGTGTGA TTAATACAGA GACTCTTACC AAGGTAAAC CTAAACAGG  
 CCTCCACACT AATTATGTCT CTGAGAATGG TTCCATTTG GATTTTGTCC  
 20701 TCAGGAAAAT GGATGGGAAA AAGATGCTAC AGAATTTTCA GATAAAAATG  
 AGTCCTTTTA CCTACCTTTT TTCTACGATG TCTTAAAGT CTATTTTAC  
 20751 AAATAAGAGT TGGAATAAT TTTGCCATGG AAATCAATCT AAATGCCAAC  
 TTTATTCTCA ACCTTTATTA AAACGGTACC TTTAGTTAGA TTTACGGTTG  
 20801 CTGTGGAGAA ATTTCTGTGA CTCCAACATA GCGCTGTATT TGCCCGACAA  
 GACACCTCTT TAAAGGACAT GAGGTGTAT CGCGACATAA ACGGGCTGTT

Figure 26 v

20851 GCTAAAGTAC AGCTCTTCCA ACGTAAAAAT TTCTGATAAC TCAAACTCTT  
 CGATTTCATG TGGGAAGGT TGCATTTTAA AAGACTATTG GGTTCGAA

20901 ACGACTACAT GAACAAGCGA GTGGTGGCTC CCGGGCTAGT GGACTGCTAC  
 TGCTGATGTA CTTGTTCGCT CACCACCGAG GGCCCGATCA CCTGACGATG

20951 ATTAACCTTG GAGCACGCTG GTCCCTTGAC TATATGGACA ACGTCAACCC  
 TAATTGGAAC CTCGTGCGAC CAGGGAACTG ATATACCTGT TGCAGTTGGG

21001 ATTTAACCAC CACCGCAATG CTGGCCTGCG CTACCGCTCA ATGTTGCTGG  
 TAAATTGGTG GTGGCGTTAC GACCGGACGC GATGGCGAGT TACAACGACC

21051 GCAATGGTCG CTATGTGCCC TTCCACATCC AGGTGCCTCA GAAGTTCTTT  
 CGTTACCAGC GATACACGGG AAGGTGTAGG TCCACGGAGT CTTCAAGAAA

21101 GCCATTAAAA ACCTCCTTCT CCTGCCGGGC TCATACACCT ACGAGTGGAA  
 CGGTAATTTT TGGAGGAAGA GGACGGCCCG AGTATGTGGA TGCTCACCTT

21151 CTTCAGGAAG GATGTTAACA TGGTTCTGCA GAGCTCCCTA GGAAATGACC  
 GAAGTCCTTC CTACAATTGT ACCAAGACGT CTCGAGGGAT CCTTTACTGG

21201 TAAGGGTTGA CGGAGCCAGC ATTAAGTTTG ATAGCATTTG CCTTTACGCC  
 ATTCCCAACT GCCTCGGTCTG TAATTCAAAC TATCGTAAAC GGAAATGCGG

21251 ACCTTCTTCC CCATGGCCCA CAACACCGCC TCCACGCTTG AGGCCATGCT  
 TGGAAGAAGG GGTACCGGGT GTTGTGGCGG AGGTGCGAAC TCCGCTACGA

21301 TAGAAACGAC ACCAACGACC AGTCCTTTAA CGACTATCTC TCCGCCGCCA  
 ATCTTTGCTG TGGTTGCTGG TCAGGAAATT GCTGATAGAG AGGCGGCGGT

21351 ACATGCTCTA CCCTATACCC GCCAACGCTA CCAACGTGCC CATATCCATC  
 TGTACGAGAT GGGATATGGG CGGTTGCGAT GGTTCACCGG GTATAGGTAG

21401 CCCTCCCGCA ACTGGGCGGC TTTCGCGGC TGGGCCTTCA CGCGCCTTAA  
 GGGAGGGCGT TGACCCGCCG AAAGGCGCCG ACCCGGAAGT GCAGCGAATT

21451 GACTAAGGAA ACCCCATCAC TGGGCTCGGG CTACGACCCT TATTACACCT  
 CTGATTCCTT TGGGGTAGTG ACCCGAGCCC GATGCTGGGA ATAATGTGGA

21501 ACTCTGGCTC TATACCCTAC CTAGATGGAA CCTTTACCT CAACCACACC  
 TGAGACCGAG ATATGGGATG GATCTACCTT GGAAAATGGA GTTGGTGTGG

21551 TTTAAGAAGG TGGCCATTAC CTTTGACTCT TCTGTGAGCT GGCCTGGCAA  
 AAATCTTCC ACCGGTAATG GAAACTGAGA AGACAGTCGA CCGGACCGTT

21601 TGACCGCCTG CTTACCCCA ACGAGTTTGA AATTAAGCGC TCAGTTGACG  
 ACTGGCGGAC GAATGGGGGT TGCTCAAAC TTAATTGCGG AGTCAACTGC

21651 GGGAGGGTTA CAACGTTGCC CAGTGTAACA TGACCAAAGA CTGGTTCTCTG  
 CCCTCCCAAT GTTGCAACGG GTCACATTGT ACTGGTTTCT GACCAAGGAC

21701 GTACAAATGC TAGCTAACTA TAACATTGGC TACCAGGGCT TCTATATCCC  
 CATGTTTACG ATCGATTGAT ATTGTAACCG ATGGTCCCGA AGATATAGGG

21751 AGAGAGCTAC AAGGACCGCA TGTACTCCTT CTTTAGAAAC TTCCAGCCCA  
 TCTCTCGATG TTCTTGGCGT ACATGAGGAA GAAATCTTTG AAGGTGGGGT

Figure 26 W

21801 TGAGCCGTCA GCTGGTGGAT GATACTAAAT ACAAGGACTA CCAACASTTG  
 ACTCGGCAGT CACACCTA CTATGATTTA TGTTCCTGAT GGTGTAC

21851 GGCATCCTAC ACCAACACAA CAACTCTGGA TTTGTGGCT ACCTTGCCCC  
 CCGTAGGATG TGGTTGTGTT GTTGAGACCT AAACAACCGA TGGAACGGGG

21901 CACCATGCGC GAAGGACAGG CCTACCCTGC TAACTTCCCC TATCCGCTTA  
 TGGTACGCG CTTCCTGTCC GGATGGGACG ATTGAAGGGG ATAGGCGAAT

21951 TAGGCAAGAC CGCAGTTGAC AGCATTACCC AGAAAAAGTT TCITTTGCGAT  
 ATCCGTTCTG GCGTCAACTG TCGTAATGGG TCTTTTTCAA AGAAAACGTA

22001 CGCACCCCTTT GCGGCATCCC ATTCTCCAGT AACTTTATGT CCATGGGCGC  
 CCGTGGGAAA CCGCGTAGGG TAAGAGGTCA TTGAAATACA GGTACCCGCG

22051 ACTCACAGAC CTGGGCCAAA ACCTTCTCTA CGCCAACTCC GCCACGCGC  
 TGAGTGTCTG GACCCGGTTT TGGAAGAGAT GCGGTTGAGG CCGGTGCGCG

22101 TAGACATGAC TTTTGAGGTG GATCCCATGG ACGAGCCAC CTTTCTTTAT  
 ATCTGTACTG AAAACTCCAC CTAGGGTACC TGCTCGGGTG GGAAGAAATA

22151 GTTTTGTGTTG AAGTCTTTGA CGTGGTCCGT GTGCACCAGC CGCACCGCGG  
 CAAAACAAAC TTCAGAACT GCACCAGGCA CACGTGGTCG GCGTGGCGCC

22201 CGTCATCGAA ACCGTGTACC TGCACGCGC CTTCTCGGCC GGCAACGCCA  
 GCAGTAGCTT TGGCACATGG ACGCGTGGG GAAGAGCCGG CCGTTGCGGT

22251 CAACATAAAG AAGCAAGCAA CATCAACAAC AGCTGCCGCC ATGGGCTCCA  
 GTGTATTTT TTCGTTCGTT GTAGTCTGTG TCGACGGCGG TACCCGAGGT

22301 GTGAGCAGGA ACTGAAAGCC ATTGTCAAAG ATCTTGTTG TGGGCCATAT  
 CACTCGTCCT TGACTTTCGG TAACAGTTT TAGAACCAAC ACCCGGTATA

22351 TTTTGGGCA CCTATGACAA GCGCTTTCCA GGCTTTGTTT CTCCACACAA  
 AAAAACCCGT GGATACTGTT CGCGAAAGGT CCGAAACAA GAGGTGTGTT

22401 GCTCGCCTGC GCCATAGTCA ATACGGCCGG TCGCGAGACT GGGGGCGTAC  
 CGAGCGGACG CCGTATCAGT TATGCCGCC AGCGCTCTGA CCCCCGCATG

22451 ACTGGATGGC CTTTGCCCTG AACC CGCACT CAAAAACATG CTACCTCTTT  
 TGACCTACCG GAAACGGACC TTGGGCGTGA GTTTTGTAC GATGGAGAAA

22501 GAGCCCTTTG GCTTTTCTGA CCAGCGACTC AAGCAGGTTT ACCAGTTTGA  
 CTCGGGAAAC CGAAAAGACT GGTGCTGAG TTCGTCCAA TGGTCAAAC

22551 GTACGAGTCA CTCCTGCGCC GTAGCGCCAT TGCTTCTTCC CCCGACCGCT  
 CATGCTCAGT GAGGACGCG CATCGCGTA ACGAAGAAG GGGCTGGCGA

22601 GTATAACGCT GGAAAAGTCC ACCCAAAGCG TACAGGGGCC CAACTCGGCC  
 CATATTGCGA CCTTTTCAGG TGGGTTTCGC ATGTCCCCG GTTGAGCCGG

22651 GCCTGTGGAC TATTCTGCTG CATGTTTCTC CACGCCCTTG CCAACTGGCC  
 CGGACACCTG ATAAGACGAC GTACAAAGAG GTGCGGAAAC GGTGACCGG

22701 CCAAACTCCC ATGGATCACA ACCCCACCAT GAACCTTATT ACCGGGGTAC  
 GGTTTGAGGG TACCTAGTGT TGGGGTGTA CTTGGAATAA TGGCCCCATG

Figure 26 x

22751 CCAACTCCAT GCTCAACAGT CCCCAGGTAC AGCCACCGAT GGGTGGGAC  
 GGTTCAGGTA CTTGTCTCA GGGGTCCATG TCGGGTGGGA CGCAGCCTG

22801 CAGGAACAGC TCTACAGCTT CCTGGAGCGC CACTCGCCCT ACTTCCGCAG  
 GTCCCTGTCTG AGATGTCGAA GGACCTCGCG GTGAGCGGGA TGAAGGCGTC

22851 CCACAGTGCG CAGATTAGGA GCGCCACTTC TTTTGTGCAC TTGAAAAACA  
 GGTGTCACGC GTCTAATCCT CGCGGTGAAG AAAACAGTG AACTTTTTGT

22901 TGTAAAAATA ATGTACTAGA GACACTTTCA ATAAAGGCAA ATGCTTTTAT  
 ACATTTTAT TACATGATCT CTGTGAAAGT TATTTCCGTT TACGAAAAA

22951 TTGTACACTC TCGGGTGATT ATTTACCCCC ACCCTTGCCG TCTGCGCCGT  
 AACATGTGAG AGCCCACTAA TAAATGGGGG TGGGAACGGC AGACGCGGCA

23001 TTAAAAATCA AAGGGGTCTT GCCGCGCATC GCTATGCGCC ACTGGCAGGG  
 AATTTTGTAGT TTCCCAAGA CGGCGCGTAG CGATACGCGG TGACCGTCCC

23051 ACACGTTGCG ATACTGGTGT TTAGTGCTCC ACTTAACTC AGGCACAACC  
 TGTGCAACGC TATGACCACA AATCACGAGG TGAATTTGAG TCCGTGTTGG

23101 ATCCGCGGCA GCTCGGTGAA GTTTTCACTC CACAGGCTGC GCACCATCAC  
 TAGGCGCCGT CGAGCCACTT CAAAAGTGAG GTGTCCGACG CGTGGTAGTG

23151 CAACGCGTTT AGCAGGTCGG GCGCCGATAT CTTGAAGTCG CAGTTGGGGC  
 GTTGCGCAAA TCGTCCAGCC CGCGGCTATA GAACTTCAGC GTCAACCCCG

23201 CTCCGCCCTG CGCGCGCGAG TTGCGATACA CAGGGTTGCA GCACTGGAAC  
 GAGGCGGGAC GCGCGCGCTC AACGCTATGT GTCCCAACGT CGTGACCTTG

23251 ACTATCAGCG CCGGGTGGTG CACGCTGGCC AGCACGCTCT TGTCGGAGAT  
 TGATAGTCGC GGCCCAACCAC GTGCGACCGG TCGTGCGAGA ACAGCCTCTA

23301 CAGATCCGCG TCCAGGTCCT CCGCGTTGCT CAGGGCGAAC GGAGTCAACT  
 GTCTAGGCGC AGGTCCAGGA GCGGCAACGA GTCCCGCTTG CCTCAGTTGA

23351 TTGGTAGCTG CCTTCCCAA AAGGGCGCGT GCCCAGGCTT TGAGTTGCAC  
 AACCATCGAC GGAAGGTTT TTCCCGCGCA CGGGTCCGAA ACTCAACGTG

23401 TCGCACCGTA GTGGCATCAA AAGGTGACCG TGCCCGGTCT GGGCGTTAGG  
 AGCGTGGCAT CACCGTAGTT TTCCACTGGC ACGGGCCAGA CCCGCAATCC

23451 ATACAGCGCC TGCATAAAAG CCTTGATCTG CTTAAAAGCC ACCTGAGCCT  
 TATGTCGCGG ACGTATTTT GGAACAGAC GAATTTTCGG TGGACTCGGA

23501 TTGCGCCTTC AGAGAAGAAC ATGCCGCAAG ACTTGCCGGA AAACGTATTG  
 AACGCGGAAG TCTCTTCTTG TACGGCGTTC TGAACGGCCT TTGACTAAC

23551 GCCGGACAGG CCGCGTCGTG CACGAGCAC CTTGCGTCGG TGTGGAGAT  
 CGGCTGTCC GCGGAGCAC GTGCGTCGTG GAACGAGCC ACAACCTCTA

23601 CTGCACCACA TTTCGGCCCC ACCGTTCTT CACGATCTTG GCCTTGCTAG  
 GACGTGGTGT AAAGCCGGGG TGGCCAAGAA GTGCTAGAAC CGGAACGATC

23651 ACTGCTCCTT CAGCGCGCGC TGCCCGTTTT CGCTCGTCAC ATCCATTTC  
 TGACGAGGAA GTCGCGCGCG ACGGGCAAAA GCGAGCAGTG TAGGTAAAGT

Figure 26Y

23701 ATCACGTGCT CATTATTTAT CATAATGCTT CCGTGTAGAC ACTTAACTC  
 TAGTGACACGA GGAATAAATA GTATTACGAA GGCACATCTG TGAATTCGAG  
 23751 GCCTTCGATC TCAGCGCAGC GGTGCAGCCA CAACGCGCAG CCCGTGGGCT  
 CGGAAGCTAG AGTCGCGTCG CCACGTCGGT GTTGC GCGTC GGGCACCCGA  
 23801 CGTGATGCTT GTAGGTCACC TCTGCAAACG ACTGCAGGTA CGCCTGCAGG  
 GCACTACGAA CATCCAGTGG AGACGTTTGC TGACGTCCAT GCGGACGTCC  
 23851 AATCGCCCCA TCATCGTCAC AAAGGTCTTG TTGCTGGTGA AGGTGAGCTG  
 TTAGCGGGGT AGTAGCAGTG TTTCCAGAAC AACGACCACT TCCAGTCGAC  
 23901 CAACCCGCGG TGCTCCTCGT TCAGCCAGGT CTTGCATACG GCCGCCAGAG  
 GTTGGGCGCC ACGAGGAGCA AGTCGGTCCA GAACGTATGC CGGCGGTCTC  
 23951 CTTCCACTTG GTCAGGCAGT AGTTTGAAGT TCGCCTTTAG ATCGTTATCC  
 GAAGGTGAAC CAGTCCGTCA TCAAACTTCA AGCGGAAATC TAGCAATAGG  
 24001 ACGTGGTACT TGTCCATCAG CGCGCGCGCA GCCTCCATGC CTTTCTCCCA  
 TGCACCATGA ACAGGTAGTC GCGCGCGCGT CGGAGGTACG GGAAGAGGGT  
 24051 CGCAGACACG ATCGGCACAC TCAGCGGGTT CATCACCGTA ATTTCACTTT  
 GCGTCTGTGC TAGCCGTGTG AGTCGCCCAA GTAGTGGCAT TAAAGTAAA  
 24101 CCGCTTCGCT GGGCTCTTCC TCTTCTCTT GCGTCCGCAT ACCACGCGCC  
 GCGGAAGCGA CCCGAGAAGG AGAAGGAGAA CGCAGGCGTA TGGTGC GCGG  
 24151 ACTGGGTCGT CTTCAATCAG CCGCCGCACT GTGCGCTTAC CTCTTTGCC  
 TGACCCAGCA GAAGTAAATC GGCGGCGTGA CACGCGAATG GAGGAAACGG  
 24201 ATGCTTGATT AGCACCGGTG GGTJGCTGAA ACCCACCATT TGTAGCGCCA  
 TACGAACATA TCCTGGCCAC CCAACGACTT TGGGTGGTAA ACATCGCGGT  
 24251 CATCTTCTCT TTCTTCCTCG CTGTCCACGA TTACCTCTGG TGATGGCGGG  
 GTAGAAGAGA AAGAAGGAGC GACAGGTGCT AATGGAGACC ACTACCGCCC  
 24301 CGCTCGGGCT TGGGAGAAGG GCGCTCTTTT TTCTTCTTGG GCGCAATGGC  
 GCGAGCCCGA ACCCTCTTCC CGCGAAGAAA AAGAAGAACC CGCGTTACCG  
 24351 CAAATCCGCC GCCGAGGTG ATGGCCGCGG GCTGGGTGTG CGCGGCACCA  
 GTTTAGGCGG CGGCTCCAGC TACCGGCGCC CGACCCACAC GCGCCGTGGT  
 24401 GCGCGTCTTG TGATGAGTCT TCCTCGTCCT CGGACTCGAT ACGCCGCCTC  
 CGCGCAGAAC ACTACTCAGA AGGAGCAGGA GCCTGAGCTA TCGGGCGGAG  
 24451 ATCCGCTTTT TTGGGGGCGC CCGGGGAGGC GCGGCGGACG GGGACGGGGA  
 TAGGCGAAAA AACCCCGCGG GGCCCCCTCG CCGCGCTGC CCCTGCCCTT  
 24501 CGACACGTCC TCCATGGTTG GGGGACGTG CGCCGCACCG CGTCCGCGCT  
 GCTGTGCAGG AGGTACCAAC CCCCTGCAGC GCGGCGTGGC GCAGGCGCGA  
 24551 CGGGGGTGGT TTCGCGCTGC TCCTCTTCCC GACTGGCCAT TTCTTCTTCC  
 GCCCCACCA AAGCGCGACG AGGAGAAGGG CTGACCGGTA AAGGAAGAGG  
 24601 TATAGGCAGA AAAAGATCAT GGAGTCAGTC GAGAAGAAGG ACAGCCTAAC  
 ATATCCGTCT TTTTCTAGTA CCTCAGTCAG CTCTTCTTCC TGTCGGATTG

Figure 262

24651 CGCCCCCTCT GTCGCCA CCACCGCCTC CACCGATGCC GCUAAGTC  
GCGGGGGAGA CTC AAGCGGT GGTGGCGGAG GTGGCTACGG CGGTTCGCG

24701 CTACCACCTT CCCCCTCGAG GCACCCCGC TTGAGGAGGA GGAAGTGATT  
GATGGTGGAA GGGGCAGCTC CGTGGGGGCG AACTCCTCCT CCTTCACTAA

24751 ATCGAGCAGG ACCCAGGTTT TGTAAAGCGAA GACGACGAGG ACCGCTCAGT  
TAGCTCGTCC TGGGTCCAAA ACATTGCTT CTGCTGCTCC TGGCGAGTCA

24801 ACCAACAGAG GATAAAAAGC AAGACCAGGA CAACGCAGAG GCAAACGAGG  
TGTTTGCTCT CTATTTTTCG TTCTGGTCTT GTTGCCTCTC CGTTTGCTCC

24851 AACAAGTCGG GCGGGGGGAC GAAAGGCATG GCGACTACCT AGATGTGGGA  
TTGTTACGCC CGCCCCCTG CTTTCCGTAC CGCTGATGGA TCTACACCTT

24901 GACGACGTGC TGTGAAGCA TCTGCAGCGC CAGTGCGCCA TTATCTGCGA  
CTGCTGCACG ACAACTTCGT AGACGTGCGG GTCACGCGGT AATAGACGCT

24951 CGCGTTGCAA GAGCGCAGCG ATGTGCCCCCT CGCCATAGCG GATGTCAGCC  
GCGCAACGTT CTCGCGTCCG TACACGGGGA GCGGTATCGC CTACAGTCGG

25001 TTGCCTACGA ACGCCACCTA TTCTCACCGC GCGTACCCCC CAAACGCCAA  
AACGGATGCT TCGGTGGAT AAGAGTGGCG CGCATGGGG GTTTGCGGTT

25051 GAAAACGGCA CATGCGAGCC CAACCCGCGC CTCAACTTCT ACCCCGTATT  
CTTTTGCCGT GTACGCTCGG GTTGGGCGCG GAGTTGAAGA TGGGGCATAA

25101 TGCCGTGCCA GAGGTGCTTG CCACCTATCA CATCTTTTTC CAAAACGCA  
ACGGCACGGT CTCCACGAAC GGTGGATAGT GTAGAAAAG GTTTTGACGT

25151 AGATACCCCT ATCCTGCCGT GCCAACCGCA GCCGAGCGGA CAAGCAGCTG  
TCTATGGGGA TAGGACGGCA CGGTGGCGT CGGCTCGCT GTTCGTCGAC

25201 GCCTTGCGGC AGGGCGCTGT CATACTGAT ATCGCCTCGC TCAACGAAGT  
CGGAACGCCG TCCGCGACA GTATGGAATA TAGCGGAGCG AGTTGCTTCA

25251 GCCAAAAATC TTTGAGGGTC TTGGACGCGA CGAGAAGCGC GCGGCAACG  
CGGTTTTTAG AAACCTCCAG AACCTGCGCT GCTCTTCGCG CGCCGTTTGC

25301 CTCTGCAACA GGAAAACAGC GAAAATGAAA GTCACCTCTGG AGTGTGGTG  
GAGACGTTGT CCTTTTGTG CTTTTACTTT CAGTGAGACC TCACAACCAC

25351 GAACTCGAGG GTGACAACGC GCGCCTAGCC GTACTAAAC GCAGCATCGA  
CTTGAGCTCC CACTGTTGCG CGCGGATCGG CATGATTTTG CGTCGTAGCT

25401 GGTCACCCAC TTTGCCTACC CGGCACTTAA CCTACCCCC AAGGTCATGA  
CCAGTGGGTG AAACGGATGG GCCGTGAATT GGATGGGGG TTCCAGTACT

25451 GCACAGTCAT GAGTGAGCTG ATCGTGCGCC GTGCGCAGCC CCTGGAGAGG  
CGTGTAGTA CTCACGAC TAGCACGCGG CACGCGTCGG GGACCTCTCC

25501 GATGCAAATT TGCAAGAACA AACAGAGGAG GGCCTACCCG CAGTTGGCGA  
CTACGTTTAA ACGTTCTGT TTGTCTCCTC CCGGATGGGC GTCAACCGCT

25551 CGAGCAGCTA GCGCGCTGGC TTCAAACGCG CGAGCCTGCC GACTTGGAGG  
GCTCGTCGAT CGCGCGACCG AAGTTTGCGC GCTCGGACCG CTGAACCTCC

Figure 26 AA



25601 AGCGACGCAA AATGATG GCCGCAGTGC TCGTTACCGT GGAGCTAG  
 TCGCTGCGTT TGATTACTAC CCGCGTCACG AGCAATGGCA CCTCGAACTC  
 25651 TGCATGCAGC GGTTCCTTGC TGACCCGGAG ATGCAGCGCA AGCTAGAGGA  
 ACGTACGTCG CCAAGAAACG ACTGGGCCTC TACGTGCGGT TCGATCTCCT  
 25701 AACATTGCAC TACACCTTTC GACAGGGCTA CGTACGCCAG GCCTGCAAGA  
 TTGTAACGTG ATGTGGAAAG CTGTCCCGAT GCATGCGGTC CGGACGTTCT  
 25751 TCTCCAACGT GGAGCTCTGC AACCTGGTCT CCTACCTTGG AATTTTGCAC  
 AGAGGTTGCA CTCGAGACG TTGGACCAGA GGATGGAACC TTAACACGTG  
 25801 GAAAACCGCC TTGGGCAAAA CGTGCTTCAT TCCACGCTCA AGGGCGAGGC  
 CTTTTGCGGG AACCCGTTTT GCACGAAGTA AGGTGCGAGT TCCCGCTCCG  
 25851 GCGCCGCGAC TACGTCCGCG ACTGCGTTTA CTTATTCTA TGCTACACCT  
 CCGGCGCGTG ATGCAGGCGC TGACGCAAAAT GAATAAAGAT ACGATGTGGA  
 25901 GGCAGACGGC CATGGGCGTT TGGCAGCAGT GCTTGGAGGA GTGCAACCTC  
 CCGTCTGCCG GTACCCGCAA ACCGTGCTCA CGAACCTCCT CACGTTGGAG  
 25951 AAGGAGCTGC AGAAACTGCT AAAGCAAAAC TTGAAGGACC TATGGACGGC  
 TTCCTCGACG TCTTTGACGA TTTGCTTTTG AACTTCCTGG ATACCTGCCG  
 26001 CTTCAACGAG CGCTCCGTGG CCGCGCACCT GCGGACATC ATTTTCCCGG  
 GAAGTTGCTC GCGAGGCACC GCGCGTGGA CCGCCTGTAG TAAAAGGGGC  
 26051 AACGCCTGCT TAAAACCTG CAACAGGGTC TGCCAGACTT CACCAGTCAA  
 TTGCGGACGA ATTTTGGGAC GTTGTCCAG ACGGTCTGAA GTGGTCAGTT  
 26101 AGCATGTTGC AGAACTTTAG GAACTTTATC CTAGAGCGCT CAGGAATCTT  
 TCGTACAACG TCTTGAAATC CTTGAAATAG GATCTCGGA GTCCCTTAGAA  
 26151 GCCCCCCACC TGCTGTGCAC TTCCTAGCGA CTTTGTGCCC ATTAAGTACC  
 CGGGCGGTGG ACGACACGTG AAGGATCGCT GAAACACGGG TAATTCATGG  
 26201 GCGAATGCCC TCCGCCGCTT TGGGGCCACT GCTACCTTCT GCAGCTAGCC  
 CGCTTACGGG AGGCGGCGAA ACCCCGGTGA CGATGGAAGA CGTCGATCGG  
 26251 AACTACCTTG CCTACCCTC TGACATAATG GAAGACGTGA GCGGTGACGG  
 TTGATGGAAC GGATGGTGAG ACTGTATTAC CTTCTGCACT CGCCACTGCC  
 26301 TCTACTGGAG TGTCCTGTC GCTGCAACCT ATGCACCCCG CACCGCTCCC  
 AGATGACCTC ACAGTGACAG CGACGTGGA TACGTGGGGC GTGGCGAGGG  
 26351 TGGTTTGCAA TTCGCAGCTG CTTAACGAAA GTCAAATTAT CGGTACCTTT  
 ACCAAACGTT AAGCGTCGAC GAATTGCTTT CAGTTTAATA GCCATGGAAA  
 26401 GAGCTGCAGG GTCCCTCGCC TGACGAAAAG TCCGCGGCTC CGGGGTGAA  
 CTCGACGTCC CAGGGAGCGG ACTGCTTTTC AGGCGCCGAG GCCCAACTT  
 26451 ACTCACTCCG GGGCTGTGGA CGTCGGCTTA CCTTCGCAAA TTTGTACCTG  
 TGAGTGAGGC CCCGACACCT GCAGCCGAAT GGAAGCGTTT AAACATGGAC  
 26501 AGGACTACCA CGCCACGAG ATTAGTTCT ACGAAGACCA ATCCCGCCCG  
 TCCTGATGGT GCGGGTGCTC TAATCCAAGA TGCTTCTGGT TAGGGCGGGC

Figure 26 AB

26551 CCTAATGCGG AATTACCGC CTGCGTCATT ACCCAGGGCC ACATTCGG  
 GGATTACGCC TCGAATGGCG GACGCAGTAA TGGGTCCCGG TGTAAGAACC  
 26601 CCAATTGCAA GCCATCAACA AAGCCCGCCA AGAGTTTCTG CTACGAAAGG  
 GGTAAACGTT CGGTAGTTGT TTCGGCGGT TCTCAAAGAC GATGCTTTCC  
 26651 GACGGGGGGT TTA CTGGAC CCCAGTCCG GCGAGGAGCT CAACCCAATC  
 CTGCCCCCA AATGAACCTG GGGGTCAGGC CGCTCCTCGA GTTGGGTTAG  
 26701 CCCCCCGCGC CGCAGCCCTA TCAGCAGCAG CCGCGGGCCC TTGCTTCCCA  
 GGGGCGGGCG GCGTCGGGAT AGTCGTCGTC GCGCCCCGGG AACGAAGGGT  
 26751 GGATGGCACC CAAAAGAAG CTGCAGCTGC CGCCGCCACC CACGGACGAG  
 CCTACCGTGG GTTTTCTTC GACGTCGACG GCGGCGGTGG GTGCCTGCTC  
 26801 GAGGAATACT GGGACAGTCA GGCAGAGGAG GTTTTGGACG AGGAGGAGGA  
 CTCCTTATGA CCCTGTCAGT CCGTCTCCTC CAAAACCTGC TCCTCCTCCT  
 26851 GGACATGATG GAAGACTGGG AGAGCCTAGA CGAGGAAGCT TCCGAGGTCTG  
 CCTGTACTAC CTTCTGACCC TCTCGGATCT GCTCCTTCGA AGGCTCCAGC  
 26901 AAGAGGTGTC AGACGAAACA CCGTCACCTT CCGTCGCATT CCCCTCGCCG  
 TTCTCCACAG TCTGCTTGT GGCAGTGGGA GCCAGCGTAA GGGGAGCGGC  
 26951 GCGCCCCAGA AATCGGCAAC CGGTTCAGC ATGGCTACAA CCTCCGCTCC  
 CGCGGGGTCT TTAGCCGTTG GCCAAGGTCG TACCGATGTT GGAGGCGAGG  
 27001 TCAGGCGCCG CCGGCACTGC CCGTTCGCGG ACCCAACCGT AGATGGGACA  
 AGTCCGCGGC GGCCGTGACG GGCAAGCGGC TGGGTGGCA TCTACCCTGT  
 27051 CCACTGGAAC CAGGGCCGGT AAGTCCAAGC AGCCGCCGCC GTTAGCCCAA  
 GGTGACCTTG GTCCCGGCCA TTCAGGTTCTG TCGGCGGCGG CAATCGGGT  
 27101 GAGCAACAAC AGCGCCAAGG CTACCGCTCA TGGCGCGGCG ACAAGAACGC  
 CTCGTTGTTG TCGCGGTTCC GATGGCGAGT ACCGCGCCCG GTTCTTGGC  
 27151 CATAGTTGCT TGCITGCAAG ACTGTGGGGG CAACATCTCC TTCGCCCCGC  
 GTATCAACGA ACGAACGTTT TGACACCCCC GTTGTAAGAGG AAGCGGGCGG  
 27201 GCTTCTTCT CTACCATCAC GGCCTGGCCT TCCCCGTA CATCCTGCAT  
 CGAAAGAAGA GATGGTAGTG CCGCACCAGA AGGGGGCATT GTAGACGTA  
 27251 TACTACCGTC ATCTCTACAG CCCATACTGC ACCGGCGGCA GCGGCAGCAA  
 ATGATGGCAG TAGAGATGTC GGGTATGACG TGGCCGCCGT CGCCGTCGTT  
 27301 CAGCAGCGGC CACACAGAAG CAAAGGCGAC CGGATAGCAA GACTCTGACA  
 GTCGTCGCGG GTGTGCTTTC GTTCCGCTG GCCTATCGTT CTGAGACTGT  
 27351 AAGCCCAAGA AATCCACAGC GCGGCGAGCA GCAGGAGGAG GAGCGCTGCG  
 TTCGGGTTCT TTAGGTGTCT CCGCCGTCGT CGTCTCTCTC CTCGCGACGC  
 27401 TCTGGCGCCC AACGAACCCG TATCGACCCG CGAGCTTAGA AACAGGATTT  
 AGACCGCGGG TTGCTTGGGC ATAGCTGGGC GCTCGAATCT TTGTCTTAA  
 27451 TTCCCACTCT GTATGCTATA TTCAACAGA GCAGGGGCCA AGAACAAGAG  
 AAGGTGAGA CATACGATAT AAAGTTGTCT CGTCCCCGGT TCTGTCTCTC

Figure 26 AC

27501 CTGAAAATAA A CAGGTC TCTGCGATCC CTCACCCGCA GCTGCCA  
 GACTTTTATT TTTGTCCAG AGACGCTAGG GAGTGGGCGT CGACGGALAT  
 27551 TCACAAAAGC GAAGATCAGC TTCGGCGCAC GCTGGAAGAC GCGGAGGCTC  
 AGTGTTCG CTTCTAGTCG AAGCCGCGTG CGACCTTCTG CGCCTCCGAG  
 27601 TCTTCAGTAA ATACTGCGCG CTGACTCTTA AGGACTAGTT TCGCGCCCTT  
 AGAAGTCATT TATGACGCGC GACTGAGAAT TCCTGATCAA AGCGCGGGAA  
 27651 TCTCAAATTT AAGCGCGAAA ACTACGTCAT CTCCAGCGGC CACACCCGGC  
 AGAGTTTAA TTCGCGCTTT TGATGCAGTA GAGGTCGCCG GTGTGGGCCG  
 27701 GCCAGCACCT GTTGTACGCG CCATTATGAG CAAGGAAATT CCCACGCCCT  
 CGGTCGTGGA CAACAGTCGC GGTAACTCTC GTTCCTTTAA GGGTGCGGGA  
 27751 ACATGTGGAG TTACCAGCCA CAAATGGGAC TTGCGGCTGG AGCTGCCCAA  
 TGTACACCTC AATGGTCGGT GTTTACCCTG AACGCGGACC TCGACGGTT  
 27801 GACTACTCAA CCCGAATAAA CTACATGAGC GCGGGACCCC ACATGATATC  
 CTGATGAGTT GGGCTTATTT GATGTACTCG CGCCCTGGGG TGTACTATAG  
 27851 CCGGGTCAAC GGAATACGCG CCCACCGAAA CCGAATTCTC CTGGAACAGG  
 GGCCAGTTG CCTTATGCGC GGGTGGCTTT GGCTTAAGAG GACCTTGTC  
 27901 CGGCTATTAC CACCACACCT CGTAATAACC TTAATCCCG TAGTTGGCCC  
 GCCGATAATG GTGGTGTGGA GCATTATTGG AATTAGGGGC ATCAACCGGG  
 27951 GCTGCCCTGG TGTACCAGGA AAGTCCCGCT CCCACCACTG TGGTACTTCC  
 CGACGGGACC ACATGGTCCT TTCAGGGCGA GGGTGGTGAC ACCATGAAGG  
 28001 CAGAGACGCC CAGGCCGAAG TTCAGATGAC TAACTCAGGG GCGCAGCTTG  
 GTCTCTGCGG GTCCGGCTTC AAGTCTACTG ATTGAGTCCC CGCGTCGAAC  
 28051 CGGGCGGCTT TCGTCACAGG GTGCGGTGCG CCGGGCAGGG TATAACTCAC  
 GCCCCCGGAA AGCAGTGTCC CACGCCAGCG GGCCCGTCCC ATATTGAGTG  
 28101 CTGACAATCA GAGGGCGAGG TATTGAGCTC AACGACGAGT CGGTGAGCTC  
 GACTGTTAGT CTCCCGCTCC ATAAGTCGAG TTGCTGCTCA GCCACTCGAG  
 28151 CTCGCTTGGT CTCCGTCCGG ACGGGACATT TCAGATCGGC GCGCCCGGCC  
 GAGCGAACCA GAGGCAGGCC TGCCCTGTAA AGTCTAGCCG CCGCGGCCGG  
 28201 GCTCTTCATT CACGCCTCGT CAGGCAATCC TAACTCTGCA GACCTCGTCC  
 CGAGAAGTAA GTGCGGAGCA GTCCGTTAGG ATTGAGACGT CTGGAGCAGG  
 28251 TCTGAGCCGC GCTCTGGAGG CATTGGAATC CTGCAATTTA TTGAGGAGTT  
 AGACTCGGCG CGAGACCTCC GTAACCTTGA GACGTTAAAT AACTCCTCAA  
 28301 TGTGCCATCG GTCTACTTTA ACCCCTTCTC GGGACCTCCC GGCCACTATC  
 ACACGGTAGC CAGATGAAAT TGGGGAAGAG CCCTGGAGGG CCGGTGATAG  
 28351 CGGATCAATT TATTCCTAAC TTTGACGCGG TAAAGGACTC GGCGGACGGC  
 GCCTAGTTAA ATAAGGATTG AAACCTGCGC ATTTCTGAG CCGCCTGCCG  
 28401 TACGACTGAA TGTTAAGTGG AGAGGCAGAG CAACTGCGCC TGAAACACCT  
 ATCTGACTT ACAATTCACC TCTCCGTCTC GTTGACGCGG ACTTTGTGGA

Figure 26 AD

28451 GGTCCACTGT CCGCCACA AGTGCTTTGC CCGCGACTCC GGTGAGTTT  
 CCAGGTGACA GCGGCGGTGT TCACGAAACG GCGCGTGAGG CCACTCAAAA  
 28501 GCTACTTTGA ATTGCCCCGAG GATCATATCG AGGGCCCCGC GCACGGCGTC  
 CGATGAAACT TAACGGGCTC CTAGTATAGC TCCCGGGCCG CGTGCCGCAG  
 28551 CGGCTTACCG CCCAGGGAGA GCTTGCCCGT AGCCTGATTG GGGAGTTTAC  
 GCCGAATGGC GGGTCCCTCT CGAACGGGCA TCGGACTAAG CCCTCAAATG  
 28601 CCAGCGCCCC CTGCTAGTTG AGCGGGACAG GGGACCCTGT GTTCTCACTG  
 GGTGCGGGG GACGATCAAC TCGCCCTGTC CCCTGGGACA CAAGAGTGAC  
 28651 TGATTTGCAA CTGTCCTAAC CCTGGATTAC ATCAAGATCT TTGTTGCCAT  
 ACTAAACGTT GACAGGATTG GGACCTAATG TAGTTCTAGA AACAACGGTA  
 28701 CTCTGTGCTG AGTATAATAA ATACAGAAAT TAAATATAC TGGGGCTCCT  
 GAGACACGAC TCATATTATT TATGTCTTTA ATTTTATATG ACCCCGAGGA  
 28751 ATCGCCATCC TGTAACGCC ACCGTCTTCA CCCGCCAAG CAAACCAAGG  
 TAGCGGTAGG ACATTTCGG TGGCAGAAGT GGGCGGGTTC GTTTGGTTCC  
 28801 CGAACCTTAC CTGGTACTTT TAACATCTCT CCCTCTGTGA TTTACAACAG  
 GCTTGGAATG GACCATGAAA ATGTAGAGA GGGAGACACT AAATGTTGTC  
 28851 TTTCAACCCA GACGGAGTGA GTCTACGAGA GAACCTCTCC GAGCTCAGCT  
 AAAGTTGGGT CTGCCTCACT CAGATGCTCT CTTGGAGAGG CTCGAGTCGA  
 28901 ACTCCATCAG AAAAAACACC ACCCTCCTTA CCTGCCGGA ACGTACGAGT  
 TGAGGTAGTC TTTTGTGG TGGGAGGAAT GGACGGCCCT TGCATGCTCA  
 28951 GCGTCACCGG CCGCTGCACC ACACCTACCG CCTGACCGTA AACCAGACTT  
 CGCAGTGGCC GCGGACGTGG TGTGGATGGC GGACTGGCAT TTGGTCTGAA  
 29001 TTTCCGGACA GACCTCAATA ACTCTGTTTA CCAGAACAGG AGGTGAGCTT  
 AAAGGCTGT CTGGAGTTAT TGAGACAAAT GGTCTGTGCC TCCACTCGAA  
 29051 AGAAAACCTT TAGGGTATTA GGCCAAAGGC GCAGCTACTG TGGGGTTTAT  
 TCTTTTGGGA ATCCATAAT CCGGTTTCCG CGTCGATGAC ACCCCAAATA  
 29101 GAACAATTCA AGCAACTCTA CGGGCTATTC TAATTCAGGT TTCTCTAGAA  
 CTTGTTAAGT TCGTTGAGAT GCGCGATAAG ATTAAGTCCA AAGAGATCTT  
 29151 TCGGGGTTGG GGTATTCTC TGTCTTGTA TTCTCTTTAT TCTTATACTA  
 AGCCCCAACC CCAATAAGAG ACAGAACT AAGAGAAATA AGAATATGAT  
 29201 ACGCTTCTCT GCCTAAGGCT CGCCGCTGTC TGTGTGCACA TTTGCATTTA  
 TGCQAAGAGA CGGATTCCGA GCGGCGGACG ACACACGTGT AAACGTAAAT  
 29251 TTGTCAGCTT TTAAACGCT GGGGTCGCCA CCCAAGATGA TTAGGTACAT  
 AACAGTCGAA AAATTTGCGA CCCCAGCGGT GGGTCTACT AATCCATGTA  
 29301 AATCCTAGGT TTACTCAGG TTGCGTCAGC CCACGGTACC ACCCAAAGG  
 TTAGGATCCA AATGAGTGGG AACGCAGTCG GGTGCCATGG TGGGTTTCC  
 29351 TGGATTTTAA GGAGCCAGCC TGTAATGTTA CATTGCGAGC TGAAGCTAAT  
 ACCTAAAT CTTGCGTCGG ACATTACAAT GTAAGCGTCG ACTTCGATTA

Figure 26 AE

29401 GAGTGCACCA CTTATAAA ATGCACCACA GAACATGAAA AGCTGCTT  
 CTCACGTGGT GAGAATATTT TACGTGGTGT CTTGTACTTT TCGACGAATA  
 29451 TCGCCACAAA AACAAAATTG GCAAGTATGC TGTATTATGCT ATTTGGCAGC  
 AGCGGTGTTT TTGTTTAAAC CGTTCATACG ACAAATACGA TAAACCGTCG  
 29501 CAGGTGACAC TACAGAGTAT AATGTTACAG TTTTCCAGGG TAAAAGTCAT  
 GTCCACTGTG ATGTCTCATA TTACAATGTC AAAAGGTCCC ATTTTCAGTA  
 29551 AAAACTTTTA TGTATACTTT TCCATTTTAT GAAATGTGCG ACATTACCAT  
 TTTTGAAAAT ACATATGAAA AGGTAAAATA CTTTACACGC TGTAAATGGTA  
 29601 GTACATGAGC AAACAGTATA AGTTGTGGCC CCCACAAAAT TGTGTGGAAA  
 CATGTACTCG TTTGTCATAT TCAACACCGG GGGTGTTTTA ACACACCTTT  
 29651 ACACTGGCAC TTTCTGCTGC ACTGCTATGC TAATTACAGT GCTCGCTTGT  
 TGTGACCGTG AAAGACGACG TGACGATACG ATTAATGTCA CGAGCGAAAC  
 29701 GTCTGTACCC TACTCTATAT TAAATACAAA AGCAGACGCA GCTTTATTGA  
 CAGACATGGG ATGAGATATA ATTTATGTTT TCGTCTGCGT CGAAATAACT  
 29751 GGAAAAGAAA ATGCCTTAAT TTAATAAGTT ACAAAGCTAA TGTCAACCACT  
 CCTTTTCTTT TACGGAATTA AATGATTCAA TGTTCGATT ACAGTGGTGA  
 29801 AACTGCTTTA CTCGCTGCTT GCAAAACAAA TTCAAAAAGT TAGCATTATA  
 TTGACGAAAT GAGCGACGAA CGTTTTGTTT AAGTTTTTCA ATCGTAATAT  
 29851 ATTAGAATAG GATTAAACC CCCCAGTCAT TTCCTGCTCA ATACCATTCC  
 TAATCTTATC CTAAATTTGG GGGGCCAGTA AAGGACGAGT TATGGTAAGG  
 29901 CCTGAACAAT TGACTCTATG TGGGATATGC TCCAGCGCTA CAACCTTGAA  
 GGACTTGTTA ACTGAGATAC ACCCTATACG AGGTCGCGAT GTTGGAACCT  
 29951 GTCAGGCTTC CTGGATGTCA GCATCTGACT TTGGCCAGCA CCTGTCCCGC  
 CAGTCCGAAG GACCTACAGT CGTAGACTGA AACCAGTCGT GGACAGGGCG  
 30001 GGATTTGTTT CAGTCCAAC ACAGCGACCC ACCCTAACAG AGATGACCAA  
 CCTAAACAAG GTCAGGTTGA TGTCGCTGGG TGGGATTGTC TCTACTGGTT  
 30051 CACAACCAAC GCGGCCGCCG CTACCGGACT TACATCTACC ACAAATACAC  
 GTGTTGGTTG CGCCGGCGGC GATGGCCTGA ATGTAGATGG TGTATTATGT  
 30101 CCCAAGTTTC TGCCTTTGTC AATAACTGGG ATAACCTGGG CATGTGGTGG  
 GGGTTCAAAG ACGGAAACAG TTATTGACCC TATTGAACCC GTACACCACC  
 30151 TTCTCCATAG CGCTTATGTT TGTATGCCTT ATTATTATGT GGCTCATCTG  
 AAGAGGTATC CGGAATACAA ACATACGGAA TAATAATACA CCGAGTAGAC  
 30201 CTGCCTAAAG CGCAAACGCG CCCGACCACC CATCTATAGT CCCATCATTG  
 GACGGATTTC GCGTTTGCGC GGGCTGGTGG GTAGATATCA GGGTAGTAAC  
 30251 TGCTACACCC AAACAATGAT GGAATCCATA GATTGGACGG ACTGAAACAC  
 ACGATGTGGG TTTGTTACTA CCTTAGGTAT CTAACCTGCC TGACTTTGTG  
 30301 ATGTTCTTTT CTCTTACAGT ATGATTAAAT GAGACATGAT TCCTCGAGTT  
 TACAAGAAAA GAGAATGTCA TACTAATTTA CTCTGTACTA AGGAGCTCAA

Figure 26 AF

30351 TTTATATTAC T CCTTGT TCGCCTTTT TGTGCGTGCT CCACAT C  
 AAATATAATG ACTGGGAACA ACGCGAAAA ACACGCACGA GGTGTAACCG  
 30401 TGC GGTTTCT CACATCGAAG TAGACTGCAT TCCAGCCTTC ACAGTCTATT  
 ACGCCAAAGA GTGTAGCTTC ATCTGACGTA AGGTCGGAAG TGTCAGATAA  
 30451 TGCTTTACGG ATTTGTCACC CTCACGCTCA TCTGCAGCCT CATCACTGTG  
 ACGAAATGCC TAAACAGTGG GAGTGCAGT AGACGTCGGA GTAGTGACAC  
 30501 GTCATCGCCT TTATCCAGTG CATTGACTGG GTCTGTGTGC GCTTTGCATA  
 CAGTAGCGGA AATAGGTCAC GTAACGACC CAGACACACG CGAAACGTAT  
 30551 TCTCAGACAC CATCCCCAGT ACAGGGACAG GACTATAGCT GAGCTTCTTA  
 AGAGTCTGTG GTAGGGGTCA TGTCCCTGTC CTGATATCGA CTGAAGAAT  
 30601 GAATTCCTTA ATTATGAAAT TTA CTGTGAC TTTCTGCTG ATTATTTGCA  
 CTTAAGAAAT TAATACTTTA AATGACACTG AAAAGACGAC TAATAACGT  
 30651 CCCTATCTGC GTTTTGTTC CCGACCTCCA AGCCTCAAAG ACATATATCA  
 GGGATAGACG CAAAACAAGG GGCTGGAGGT TCGGAGTTTC TGTATATAGT  
 30701 TGCAGATTCA CTCGTATATG GAATATTCCA AGTTGCTACA ATGAAAAAAG  
 ACGTCTAAGT GAGCATATAC CTTATAAGGT TCAACGATGT TACTTTTTTC  
 30751 CGATCTTTCC GAAGCCTGGT TATATGCAAT CATCTCTGTT ATGGTGTTC  
 GCTAGAAAGG CTTGCGACCA ATATACGTTA GTAGAGACAA TACCACAAGA  
 30801 GCAGTACCAT CTTAGCCCTA GCTATATATC CCTACCTTGA CATTGGCTGG  
 CGTCATGGTA GAATCGGGAT CGATATATAG GGATGGAAC GTAAACCGACC  
 30851 AACGCAATAG ATGCCATGAA CCACCCAACT TTCCCCGCGC CCGCTATGCT  
 TTGCGTTATC TACGGTACTT GGTGGGTGA AAGGGGCGCG GCGGATACGA  
 30901 TCCACTGCAA CAAGTTGTTG CCGGCGGCTT TGTCCCAGCC AATCAGCCTC  
 AGGTGACGTT GTTCAACAAC GGCCGCCGAA ACAGGGTCGG TTAGTCGGAG  
 30951 GCCCACCTTC TCCCACCCCT ACTGAAATCA GCTACTTTAA TCTAACAGGA  
 CGGGTGGAAG AGGGTGGGGG TGACTTTAGT CGATGAAATT AGATTGTCCT  
 31001 GGAGATGACT GACACCCTAG ATCTAGAAAT GGACGGAATT ATTACAGAGC  
 CCTCTACTGA CTGTGGGATC TAGATCTTTA CCTGCCTTAA TAATGTCTCG  
 31051 AGCGCCTGCT AGAAAGACGC AGGGCAGCGG CCGAGCAACA GCGCATGAAT  
 TCGCGGACGA TCTTTCTGCG TCCCGTCGCC GGCTCGTTGT CCGTACTTA  
 31101 CAAGAGCTCC AAGACATGGT TAACTTGCAC CAGTGCAAAA GGGGTATCTT  
 GTTCTCGAGG TTCTGTACCA ATTGAACGTG CTCACGTTTT CCCCATAGAA  
 31151 TTGTCTCGTA AAGCAGGCCA AAGTCACCTA CGACAGTAAT ACCACCGGAC  
 AACAGAGCAT TTCGTCCGGT TTCAGTGGAT GCTGTCATTA TGGTGGCCTG  
 31201 ACCGCCTTAG CTACAAGTTG CCAACCAAGC GTCAGAAATT GGTGGTCATG  
 TGGCGGAATC GATGTTCAAC GGTGTTTCG CAGTCTTTAA CCACCACTAC  
 31251 GTGGGAGAAA AGCCCATAC CATAACTCAG CACTCGGTAG AAACCGAAGG  
 CACCTCTTT TCGGTAATG GTATTGAGTC GTGAGCCATC TTTGGCTTCC

Figure 26 AG

31301 CTGCATTAC TCTTGTG AAGGACCTGA GGATCTCTGC ACCCTTCTA  
GACGTAAGTG AGTGGAACAG TTCCTGGACT CCTAGAGACG TGGGAATAT

31351 AGACCCTGTG CGGTCTCAA GATCTTATTC CCTTTAACTA ATAAAAAAA  
TCTGGGACAC GCCAGAGTTT CTAGAATAAG GGAAATTGAT TATTTTTTTT

31401 ATAATAAAGC ATCACTTACT TAAAATCAGT TAGCAAATTT CTGTCCAGTT  
TATTATTTCG TAGTGAATGA ATTTTAGTCA ATCGTTTAAA GACAGGTCAA

31451 TATTCAGCAG CACCTCCTTG CCTCCTCCC AGCTCTGGTA TTGCAGCTTC  
ATAAGTCGTC GTGGAGGAAC GGGAGGAGGG TCGAGACCAT AACGTCGAAG

31501 CTCCTGGCTG CAACTTTCT CCACAATCTA AATGGAATGT CAGTTTCCTC  
GAGGACCGAC GTTGAAAGA GGTGTTAGAT TTACCTTACA GTCAAAGGAG

31551 CTGTTCTGT CCATCCGCAC CCACTATCTT CATGTTGTTG CAGATGAAGC  
GACAAGGACA GGTAGGCGTG GGTGATAGAA GTACAACAAC GTCTACTTCG

31601 GCGCAAGACC GTCTGAAGAT ACCTTCAACC CCGTGTATCC ATATGACACG  
CCGCTTCTGG CAGACTTCTA TGGAAGTTGG GGCACATAGG TATACTGTGC

31651 GAAACCGGTC CTCCAAGTGT GCCTTTTCTT ACTCCTCCCT TTGTATCCCC  
CTTTGGCCAG GAGGTTGACA CGGAAAAGAA TGAGGAGGGA AACATAGGGG

31701 CAATGGGTTT CAAGAGAGTC CCCCTGGGGT ACTCTCTTTG CGCCTATCCG  
GTTACCCAAA GTTCTCTCAG GGGGACCCCA TGAGAGAAAC GCGGATAGGC

31751 AACCTCTAGT TACCTCCAAT GGCATGCTTG CGCTCAAAAT GGGCAACGGC  
TTGGAGATCA ATGGAGGTTA CCGTACGAAC GCGAGTTTAA CCCGTTGCCG

31801 CTCTCTCTGG ACGAGGCCGG CAACCTTACC TCCCAAAATG TAACCACTGT  
GAGAGAGACC TGCTCCGGCC GTTGGAAATGG AGGGTTTAC ATTGGTGACA

31851 GAGCCACCT CTCAAAAAA CCAAGTCAA CATAAACCTG GAAATATCTG  
CTCGGGTGGA GAGTTTTTTT GGTTCAGTTT GTATTTGGAC CTTTATAGAC

31901 CACCCCTCAC AGTTACCTCA GAAGCCCTAA CTGTGGCTGC CGCCGCACCT  
GTGGGGAGTG TCAATGGAGT CTTCGGGATT GACACCGACG GCGGCGTGGA

31951 CTAATGGTCG CGGGCAACAC ACTCACCATG CAATCACAGG CCCCGCTAAC  
GATTACCAGC GCCCGTTGTG TGAGTGGTAC GTTAGTGTCC GGGGCGATTG

32001 CGTGCACGAC TCCAACTTA GCATTGCCAC CCAAGGACCC CTCACAGTGT  
GCACGTGCTG AGGTTTGAAT CGTAACGGTG GGTTCCTGGG GAGTGTGACA

32051 CAGAAGGAAA GCTAGCCCTG CAAACATCAG GCCCCCTCAC CACCACCGAT  
GTC'TTCCTTT CGATCGGGAC GTTTGTAGTC CGGGGGAGTG GTGGTGGCTA

32101 AGCAGTACCC TTAATATCAC TGCCCTACCC CCTCTAACTA CTGCCACTGG  
TCGTCATGGG AATGATAGTG ACGGAGTGGG GGAGATTGAT GACGGTGACC

32151 TAGCTTGGGC ATTGACTTGA AAGAGCCCAT TTATACACAA AATGGAAAAC  
ATCGAACCCG TAACTGAACT TTCTCGGSTA AATATGTGTT TTACCTTTTG

32201 TAGGACTAAA GTACGGGGCT CCTTTGCATG TAACAGACGA CCTAAACACT  
ATCTGATTT CATGCCCGA GGAAACGTAC ATTGTCTGCT GGATTGTGA

Figure 26 AH

32251 TTGACCGTAG CTGGTCC AGGTGTGACT ATTAATAATA CTTCCTTCA  
 AACTGGCATC GACCAGG TCCACACTGA TAATTATTAT GAAGGAGT  
 32301 AACTAAAGTT ACTGGAGCCT TGGGTTTTGA TTCACAAGGC AATATGCAAC  
 TTGATTTCAA TGACCTCGGA ACCCAAACT AAGTGTTCG TTATACGTTG  
 32351 TTAATGTAGC AGGAGGACTA AGGATTGATT CTCAAAACAG ACGCCTTATA  
 AATTACATCG TCCTCCTGAT TCCTAACTAA GAGTTTGTG TCGGAATAT  
 32401 CTTGATGTTA GTTATCCGTT TGATGCTCAA AACCAACTAA ATCTAAGACT  
 GAACTACAAT CAATAGGCAA ACTACGAGTT TTGGTTGATT TAGATTCTGA  
 32451 AGGACAGGGC CCTCTTTTAA TAACTCAGC CCACAACTTG GATATTAAC  
 TCCTGTCCCG GGAGAAAAAT ATTTGAGTCG GGTGTGAAC CTATAATTGA  
 32501 ACAACAAAGG CCTTTACTTG TTTACAGCTT CAAACAATTC CAAAAAGCTT  
 TGTGTTTCC GGAAATGAAC AAATGTCGAA GTTGTGAAG GTTTTTCGAA  
 32551 GAGGTAAACC TAAGCACTGC CAAGGGGTTG ATGTTTGACG CTACAGCCAT  
 CTCCAATTGG ATTCGTGACG GTTCCCAAC TACAACTGC GATGTCGGTA  
 32601 AGCCATTAAT GCAGGAGATG GGCTTGAATT TGGTTCACCT AATGCACCAA  
 TCGGTAATTA CGTCTCTAC CCGAACTTAA ACCAAGTGGG TTACGTGGTT  
 32651 ACACAAATCC CCTCAAAACA AAAATTGGCC ATGGCCTAGA ATTTGATTCA  
 TGTGTTTAGG GGAGTTTGT TTTAACCAG TACCGGATCT TAACTAAGT  
 32701 AACAAAGCTA TGGTTCCTAA ACTAGGAACT GGCCTTAGTT TTGACAGCAC  
 TTGTTCCGAT ACCAAGGATT TGATCCTTGA CCGGAATCAA AACTGTCGTG  
 32751 AGGTGCCATT ACAGTAGGAA AAAAAATAA TGATAAGCTA ACTTTGTGGA  
 TCCACGGTAA TGTCATCCTT TGTTTTATT ACTATTCGAT TGAACACCT  
 32801 CCACACCAGC TCCATCTCCT AACTGTAGAC TAAATGCAGA GAAAGATGCT  
 GGTGTGGTCG AGGTAGAGGA TTGACATCTG ATTTACGTCT CTTTCTACGA  
 32851 AAACCTCACTT TGGTCTTAAC AAAATGTGGC AGTCAAATAC TTGCTACAGT  
 TTTGAGTGAA ACCAGAATTG TTTTACACCG TCAGTTTATG AACGATGTCA  
 32901 TTCAGTTTGG GCTGTTAAAG GCAGTTTGGC TCCAAATATCT GGAACAGTTC  
 AAGTCAAAAC CGACAATTTC CGTCAAACCG AGGTTATAGA CCTGTCAAG  
 32951 AAAGTGCTCA TCTTATTATA AGATTGACG AAAATGGAGT GCTACTAAAC  
 TTTACGAGT AGAATAATAT TCTAACTGC TTTTACCTCA CGATGATTG  
 33001 AATTCCTTCC TGGACCCAGA ATATTGGAAC TTTAGAAATG GAGATCTTAC  
 TTAAGGAAGG ACCTGGGTCT TATAACCTTG AAATCTTTAC CTCTAGAATG  
 33051 TGAAGGCACA GCCTATACAA ACGCTGTTGG ATTTATGCCT AACCTATCAG  
 ACTTCCGTGT CGGATATGTT TCGGACAACC TAAATACGGA TTGGATAGTC  
 33101 CTTATCCAAA ATCTCACGGT AAACTGCCA AAAGTAACAT TGTCAGTCAA  
 GAATAGGTTT TAGAGTGCCA TTTTGACGGT TTTTATTGTA ACAGTCAGTT  
 33151 GTTTACTTAA ACGGAGACAA AACTAAACCT GTAACACTAA CCATTACACT  
 CAAATGAATT TGCCTCTGTT TTGATTGGA CATGTGATG GGTAAATGTGA

Figure 26 AI



33201 AAACGGTACA CAAACAG GAGACACAAC TCCAAGTGCA TACTCTTCT  
TTTGCCATGT GTCTTTGTC CTCTGTGTTG AGGTTCACGT ATGAGATCA

33251 CATTTTCATG GGA CTGTTCT GGCACAACT ACATTAATGA AATATTGCC  
GTAAAAGTAC CCTGACCAGA CCGGTGTTGA TGTAACTACT TTATAACGG

33301 ACATCCTCTT ACACCTTTTC ATACATTGCC CAAGAATAAA GAATCGTTTG  
TGTAGGAGAA TGTGAAAAAG TATGTAACGG GTTCTTATTT CTTAGCAAAC

33351 TGTATGTTT CAACGTGTTT ATTTTCAAT TGCAGAAAT TTCAAGTCAT  
ACAATACAAA GTTGACAAA TAAAAAGTTA ACGTCTTTTA AAGTTCAGTA

33401 TTTTCATTCA GTAGTATAGC CCCACCACCA CATAGCTTAT ACAGATCACC  
AAAAGTAAGT CATCATATCG GGGTGGTGGT GTATCGAATA TGTCTAGTGG

33451 GTACCTTAAT CAAACTCACA GAACCTAGT ATTCAACCTG CCACCTCCCT  
CATGGAATTA GTTGAGTGT CTGGGATCA TAAGTTGGAC GGTGGAGGGA

33501 CCCAACACAC AGAGTACACA GTCCTTTCTC CCCGGCTGGC CTTAAAAAGC  
GGGTGTGTG TCTCATGTGT CAGGAAAGAG GGGCCGACCG GAATTTTTCG

33551 ATCATATCAT GGGTAACAGA CATATTCTTA GGTGTTATAT TCCACACGGT  
TAGTATAGTA CCCATTGTCT GTATAAGAAT CCACAATATA AGGTGTGCCA

33601 TTCCTGTGCA GCCAAACGCT CATCAGTGAT ATTAATAAAC TCCCCGGGCA  
AAGGACAGCT CGGTTTGCGA GTAGTACTA TAATTATTG AGGGGCCCGT

33651 GCTCACTTAA GTTCATGTCG CTGTCCAGCT GCTGAGCCAC AGGCTGCTGT  
CGAGTGAATT CAAGTACAGC GACAGGTCGA CGACTCGGTG TCCGACGACA

33701 CCAACTTGCG GTTGCTTAAC GGGCGGCGAA GGAGAAGTCC ACGCCTACAT  
GGTTGAACGC CAACGAATTG CCCGCCGCTT CCTCTTCAGG TCGGGATGTA

33751 GGGGGTAGAG TCATAATCGT GCATCAGGAT AGGGCGGTGG TGCTGCAGCA  
CCCCATCTC AGTATTAGCA CGTAGTCCTA TCCCCCACC ACGACGTCGT

33801 GCGCGCGAAT AACTGCTGC CGCCGCCGCT CCGTCCTGCA GGAATACAAC  
CGCGCGCTTA TTGACGACG GCGCGGCGA GGCAGGACGT CCTTATGTTG

33851 ATGGCAGTGG TCTCCTCAGC GATGATTGCG ACCGCCCCGA GCATAAGGCG  
TACCGTCACC AGAGGAGTCG CTAATAAGCG TGGCGGGCGT CGTATTCCGC

33901 CCTTGTCCTC CGGGCACAGC AGCGCACCCCT GATCTCACTT AAATCAGCAC  
GGAACAGGAG GCCCGTGTG TCGCGTGGGA CTAGAGTGAA TTAGTCTGTG

33951 AGTAACTGCA GCACAGCACC ACAATATTGT TCAAAATCCC ACAGTGCAAG  
TCATTGACGT CGTGTCTGTG TGTATAACA AGTTTATAGG TGTCACGTTC

34001 GCGCTGTATC CAAAGCTCAT GGGGGGGACC ACAGAACCCA CGTGGCCATC  
CGCGACATAG GTTTCGAGTA CCGCCCTGG TGTCTTGGT GCACCGGTAG

34051 ATACCACAAG CGCAGGTAGA TTAAGTGGCG ACCCCTCATA AACACGCTGG  
TATGGTGTTC GCGTCCATCT AATTCACCGC TGGGGAGTAT TTGTGCGACC

34101 ACATAAACAT TACCTCTTTT GGCATGTTGT AATTCACCAC CTCCCGGTAC  
TGTATTTGTA ATGAGAAAA CCGTACAACA TTAAGTGGTG GAGGGCCATG

Figure 26 AJ

34151 CATATAAACC TGGATTAAA CATGGCGCCA TCCACCACCA TCCTAATGCA  
 GTATATTGTTG AACTAATTT GTACCGCGGT AGGTGGTGGT AGGATTGCT

34201 GCTGGCCAAA ACCTGCCCCG CGGCTATACA CTGCAGGGAA CCGGGACTGG  
 CGACCGGTTT TGGACGGGCG GCCGATATGT GACGTCCCTT GGCCCTGACC

34251 AACAAATGACA GTGGAGAGCC CAGGACTCGT AACCATGGAT CATCATGCTC  
 TTGTTACTGT CACCTCTCGG GTCCTGAGCA TTGGTACCTA GTAGTACGAG

34301 GTCATGATAT CAATGTTGGC ACAACACAGG CACACGTGCA TACACTTCCT  
 CAGTACTATA GTTACAACCG TGTGTGTCC GTGTGCACGT ATGTGAAGGA

34351 CAGGATTACA AGCTCCTCCC GCGTTAGAAC CATATCCCAG GGAACAACCC  
 GTCCTAATGT TCGAGGAGGG CGCAATCTTG GTATAGGGTC CCTTGTGGG

34401 ATTCCCTGAAT CAGCGTAAAT CCCACACTGC AGGGAAGACC TCGCACGTAA  
 TAAGGACTTA GTCGCATTTA GGGTGTGACG TCCCTTCTGG AGCGTGCATT

34451 CTCACGTTGT GCATTGTCAA AGTGTTACAT TCGGGCAGCA GCGGATGATC  
 GAGTGAACA CGTAACAGTT TCACAATGTA AGCCCGTCGT CGCCTACTAG

34501 CTCCAGTATG GTAGCGCGGG TTTCTGTCTC AAAAGGAGGT AGACGATCCC  
 GAGGTCATAC CATCGCGCCC AAAGACAGAG TTTTCCTCCA TCTGCTAGGG

34551 TACTGTACGG AGTGCGCCGA GACAACCGAG ATCGTGTTGG TCGTAGTGTG  
 ATGACATGCC TCACGCGGCT CTGTTGGCTC TAGCACAACC AGCATCACAG

34601 ATGCCAAATG GAACGCCGGA CGTAGTCATA TTTCCCTGAAG CAAAACCAGG  
 TACGGTTTAC CTTGCGGCCT GCATCAGTAT AAAGGACTTC GTTTTGGTCC

34651 TCGGGGCGTG ACAAACAGAT CTGCGTCTCC GGTCTCGCCG CTTAGATCGC  
 ACGCCCGCAC TGTGTGTCTA GACGCAGAGG CCAGAGCGGC GAATCTAGCG

34701 TCTGTGTAGT AGTTGTAGTA TATCCACTCT CTCAAAGCAT CCAGGCGCCC  
 AGACACATCA TCAACATCAT ATAGGTGAGA GAGTTTCGTA GGTCCGCGGG

34751 CCTGGCTTCG GGTCTATGT AAACCTCTTC ATGCGCCGCT GCCCTGATAA  
 GGACCGAAGC CCAAGATACA TTTGAGGAAG TACGCGGCGA CGGGACTATT

34801 CATCCACCAC CGCAGAATAA GCCACACCCA GCCAACCTAC ACATTGCTTC  
 GTAGGTGGTG GCGTCTTATT CGGTGTGGGT CGGTTGGATG TGTAAGCAAG

34851 TGCGAGTCAC ACACGGGAGG AGCGGGAAGA GCTGGAAGAA CCATGTTTTT  
 ACGCTCAGTG TGTGCCCTCC TCGCCCTTCT CGACCTTCTT GGTACAAAAA

34901 TTTTTTATTC CAAAAGATTA TCCAAAACCT CAAAATGAAG ATCTATTAAG  
 AAAAAATAAG GTTTTCTAAT AGGTTTTGGA GTTTTACTTC TAGATAATTC

34951 TGAACGCGCT CCCCTCCGGT GGCCTGGTCA AACTCTACAG CCAAAGAACA  
 ACTTGCCGGA GGGGAGGCCA CCGCACCAGT TTGAGATGTC GGTTCCTTGT

35001 GATAATGGCA TTTGTAAGAT GTTGACAAAT GGCTTCCAAA AGGCAAACGG  
 CTATTACCGT AAACATTCTA CAACGTGTTA CCGAAGGTTT TCCGTTTGCC

35051 CCCTCACGTC CAAGTGGACG TAAAGGCTAA ACCCTTCAGG GTGAATCTCC  
 GGGAGTGCAG GTTCACCTGC ATTTCCGATT TGGGAAGTCC CACTAGAGG

Figure 26 AK

35101 TCTATAAACA TTAGCACC TTCAACCATG CCCAAATAAT TCTCAT G  
 AGATATTGT AAGGTCGTGG AAGTTGGTAC GGGTTTATTA AGAGTAGAGC  
 35151 CCACCTTCTC AATATATCTC TAAGCAAATC CCQAATATTA AGTCCGGCCA  
 GGTGGAAGAG TTATATAGAG ATTCTGTTAG GGCTTATAAT TCAGGCCGGT  
 35201 TTGTAAAAAT CTGCTCCAGA GCGCCCTCCA CCTTCAGCCT CAAGCAGCGA  
 AACATTTTAA GACGAGGTCT CGCGGGAGGT GGAAGTCGGA GTTCGTCGCT  
 35251 ATCATGATTG CAAAAATTCA GGTTCCTCAC AGACCTGTAT AAGATTCAAA  
 TAGTACTAAC GTTTTAAAGT CCAAGGAGTG TCTGGACATA TTCTAAGTTT  
 35301 AGCGGAACAT TAACAAAAAT ACCGCGATCC CGTAGGTCCC TTCGCAGGGC  
 TCGCCTTGTA ATTGTTTTTA TGGCGCTAGG GCATCCAGGG AAGCGTCCCG  
 35351 CAGCTGAACA TAATCGTGCA GGTCTGCACG GACCAGCGCG GCCACTTCCC  
 GTCGACTTGT ATTAGCACGT CCAGACGTGC CTGGTCGCGC CGGTGAAGGG  
 35401 CGCCAGGAAC CATGACAAAA GAACCCACAC TGATTATGAC ACGCATACTC  
 CGGCTCCTTG GTACTGTTTT CTTGGGTGTG ACTAATACTG TCGGTATGAG  
 35451 GGAGCTATGC TAACCAGCGT AGCCCCGATG TAAGCTTGTT GCATGGGCGG  
 CCTCGATACG ATTGGTCGCA TCGGGGCTAC ATTCTGAACA CGTACCCGCC  
 35501 CGATATAAAA TGCAAGGTGC TGCTCAAAAA ATCAGGCAAA GCCTCGCGCA  
 GCTATATTTT ACGTTCACG ACGAGTTTTT TAGTCCGTTT CGGAGCGCGT  
 35551 AAAAAGAAAG CACATCGTAG TCATGCTCAT GCAGATAAAG GCAGGTAAGC  
 TTTTCTTTC GTGTAGCATC AGTACGAGTA CGTCTATTTT CGTCCATTCTG  
 35601 TCCGGAACCA CCACAGAAAA AGACACCATT TTTCTCTCAA ACATGTCTGC  
 AGGCCTTGGT GGTGCTTTTT TCTGTGGTAA AAAGAGAGTT TGTACAGACG  
 35651 GGGTTTCTGC ATAAACACAA AATAAAATAA CAAAAAACA TTAAACATT  
 CCCAAAGACG TATTGTGTTT TTATTTTATT GTTTTTTTGT AAATTTGTAA  
 35701 AGAAGCCTGT CTTACAACAG GAAAAACAAC CCTTATAAGC ATAAGACGGA  
 TCTTCGGACA GAATGTTGTC CTTTTGTGTG GGAATATTCG TATTCTGCCT  
 35751 CTACGGCCAT GCCGGCGTGA CCGTAAAAAA ACTGGTCACC GTGATTAAAA  
 GATGCCGGTA CGGCCGCACT GGCATTTTTT TGACCAGTGG CACTAATTTT  
 35801 AGCACCACCG ACAGCTCCTC GGTCTGTGCC GGAGTCATAA TGTAAGACTC  
 TCGTGGTGGC TGTCGAGGAG CCACTACAGG CCTCAGTATT ACATTCTGAG  
 35851 GGTAAACACA TCAGGTTGAT TCACATCGGT CAGTCTAAA AAGCGACCGA  
 CCATTTGTGT AGTCCAACATA AGTGTAGCCA GTCACGATTT TCGCTGGCT  
 35901 AATAGCCCGG GGAATACAT ACCCGCAGGC GTAGAGACAA CATTACAGCC  
 TTATCGGGCC CCCTTATGTA TGGGCGTCCG CATCTCTGTT GTAATGTCGG  
 35951 CCCATAGGAG GTATAACAAA ATTAATAGGA GAGAAAAACA CATAAACACC  
 GGGTATCCTC CATATTGTTT TAATTATCCT CTCTTTTGTG GTATTTGTGG  
 36001 TGAAAAACCC TCCTGCCTAG GCAAAATAGC ACCCTCCCGC TCCAGAACAA  
 ACTTTTTGGG AGGACGGATC CGTTTTATCG TGGGAGGGCG AGGTCTTGTT

Figure 26 AL

36051 CATACAGCGC TACAGCG GCAGCCATAA CAGTCAGCCT TACCAG LA  
 GTATGTGCGC AAGGTGTCGC CGTCGGTATT GTCAGTCGGA ATGGTCATTT  
 36101 AAAGAAAACC TATTAAAAA ACACCACTCG ACACGGCACC AGCTCAATCA  
 TTTCTTTTGG ATAATTTTTT TGTGGTGAGC TGTGCCGTGG TCGAGTTAGT  
 36151 GTCACAGTGT AAAAAAGGGC CAAGTGCAGA GCGAGTATAT ATAGGACTAA  
 CAGTGTCA CA TTTTTCCTCG GTTCACGTCT CGCTCATATA TATCCTGATT  
 36201 AAAATGACGT AACGGTTAAA GTCCACAAA AACACCCAGA AAACCGCACG  
 TTTTACTGCA TTGCCAATTT CAGGTGTTTT TTGTGGGTCT TTTGGCGTGC  
 36251 CGAACCTACG CCCAGAAACG AAAGCCAAA AACCCACAAC TTCTCAAAT  
 GCTTGGATGC GGGTCTTTCG TTTCGGTTTT TTGGGTGTTG AAGGAGTTTA  
 36301 CGTCACTTCC GTTTTCCAC GTTACGTCAC TTCCCATTTT AAGAAAATA  
 GCAGTGAAGG CAAAAGGGTG CAATGCAGTG AAGGGTAAAA TTCTTTTGAT  
 36351 CAATTCCCAA CACATACAAG TTA CTCCGCC CTAAAACCTA CGTCACCCGC  
 GTTAAGGGTT GTGTATGTTT AATGAGGCGG GATTTTGGAT GCAGTGGGCG  
 36401 CCCGTTCCTA CGCCCCGCGC CACGTCACAA ACTCCACCCC CTCATTATCA  
 GGGCAAGGGT GCGGGGCGCG GTGCAGTGTT TGAGGTGGGG GAGTAATAGT  
 PacI  
 -----  
 36451 TATTGGCTTC AATCCAAAAT AAGGTATATT ATTGATGATG TTAATTAAGA  
 ATAACCGAAG TTAGGTTTTA TTCCATATAA TAACTACTAC AATTAATTCT  
 36501 ATTCGGATCT GCGACGCGAG GCTGGATGGC CTTCCTCATT ATGATTCTTC  
 TAAGCCTAGA CGCTGCGCTC CGACCTACCG GAAGGGGTAA TACTAAGAAG  
 36551 TCGCTTCCGG CGGCATCGGG ATGCCCGCGT TGCAGGCCAT GCTGTCCAGG  
 AGCGAAGGCC GCCGTAGCCC TACGGGCGCA ACGTCCGGTA CGACAGGTCC  
 36601 CAGGTAGATG ACGACCATCA GGGACAGCTT CAAGGCCAGC AAAAGGCCAG  
 GTCCATCTAC TGCTGGTAGT CCCTGTCGAA GTTCCGGTCG TTTTCCGGTC  
 36651 GAACCGTAAA AAGGCCGCGT TGCTGGCGTT TTTCATAGG CTCCGCCCCC  
 CTTGGCATTT TTCCGGCGCA ACGACCGCAA AAAGGTATCC GAGGCGGGGG  
 36701 CTGACGAGCA TCACAAAAAT CGACGCTCAA GTCAGAGGTG GCGAAACCGG  
 GACTGCTCGT AGTGTTTTTA GCTGCGAGTT CAGTCTCCAC CGCTTTGGGC  
 36751 ACAGGACTAT AAAGATACCA GCGCTTTCCC CCTGGAAGCT CCCTCGTGCG  
 TGTCTTGATA TTCTATGGT CCGCAAAGGG GGACCTTCGA GGGAGCACGG  
 36801 CTCTCCTGTT CCGACCTGCG CGCTTACCGG ATACCTGTCC GCCTTTCTCC  
 GAGAGGACAA GGCTGGGACG GCGAATGGCC TATGGACAGG CGGAAAGAGG  
 36851 CTTCGGGAAG CGTGCGCTT TCTCATAGCT CACGCTGTAG GTATCTCAGT  
 GAAGCCCTTC GCACCGCGAA AGAGTATCGA GTGCGACATC CATAGAGTCA  
 36901 TCGGTGTAGG TCGTTGCTC CAAGCTGGGC TGTGTGCACG AACCCCCCGT  
 AGCCACATCC AGCAAGCGAG GTTCGACCCG ACACACGTGC TTGGGGGGCA

Figure 26 AM

36951 TCAGCCCGAC GCGCGCCT TATCCGGTAA CTATCGTCTT GAGTCGCTC  
 AGTCGGGCTG GCGACGCGGA ATAGGCCATT GATAGCAGAA CTCAGGTTGG

37001 CGGTAAGACA CGACTTATCG CCACTGGCAG CAGCCACTGG TAACAGGATT  
 GCCATTCTGT GCTGAATAGC GGTGACCGTC GTCGGTGACC ATTGTCCTAA

37051 AGCAGAGCGA GGTATGTAGG CGGTGCTACA GAGTTCTTGA AGTGGTGGCC  
 TCGTCTCGCT CCATACATCC GCCACGATGT CTCAAGAACT TCACCACCGG

37101 TAACTACGGC TACACTAGAA GGACAGTATT TGGTATCTGC GCTCTGCTGA  
 ATTGATGCCG ATGTGATCTT CCTGTCATAA ACCATAGACG CGAGACGACT

37151 AGCCAGTTAC CTTCCGAAAA AGAGTTGGTA GCTCTTGATC CGGCAAACAA  
 TCGGTCAATG GAAGCCTTTT TCTCAACCAT CGAGAACTAG GCCGTTTGTT

37201 ACCACCGCTG GTAGCGGTGG TTTTCTTGTT TGCAAGCAGC AGATTACCGG  
 TGGTGGCGAC CATCGCCACC AAAAAACAA ACGTTCGTCG TCTAATGCGC

37251 CAGAAAAAAA GGATCTCAAG AAGATCCTTT GATCTTTTCT ACGGGGCTCG  
 GTCTTTTTTT CCTAGAGTTC TTCTAGGAAA CTAGAAAAGA TGCCCCAGAC

37301 ACGCTCAGTG GAACGAAAC TCACGTTAAG GGATTTTGGT CATGAGATTA  
 TCGGAGTCAC CTTGCTTTTG AGTGCAATTC CCTAAAACCA GTACTCTAAT

37351 TCAAAAAGGA TCTTCACCTA GATCCTTTTA AATCAATCTA AAGTATATAT  
 AGTTTTTCTT AGAAGTGGAT CTAGGAAAAT TTAGTTAGAT TTCATATATA

37401 GAGTAAACTT GGTCTGACAG TTACCAATGC TTAATCAGTG AGGCACCTAT  
 CTCATTTGAA CCAGACTGTC AATGGTTACG AATTAGTCAC TCCGTGGATA

37451 CTCAGCGATC TGTCTATTTT GTTCATCCAT AGTTGCCTGA CTCCCCGTCG  
 GAGTCGCTAG ACAGATAAAG CAAGTAGGTA TCAACGGACT GAGGGGCAGC

37501 TGTAGATAAC TACGATACGG GAGGGCTTAC CATCTGGCCC CAGTGCTGCA  
 ACACTTATTG ATGCTATGCC CTCCCGAATG GTAGACCGGG GTCACGACGT

37551 ATGATACCGC GAGACCCACG CTCACCGGCT CCAGATTAT CAGCAATAAA  
 TACTATGGCG CTCTGGGTGC GAGTGGCCGA GGTCTAAATA GTCGTTATTT

37601 CCAGCCAGCC GGAAGGGCCG AGCGCAGAAG TGGTCCTGCA ACTTTATCCG  
 GGTGGGTCCG CCTTCCCGGC TCGCGTCTTC ACCAGGACGT TGAAATAGGC

37651 CCTCCATCCA GTCTATTAAT TGTTGCCGGG AAGCTAGAGT AAGTAGTTCG  
 GGAGGTAGGT CAGATAATTA ACAACGGCCC TTCGATCTCA TTCATCAAGC

37701 CCAGTTAATA GTTTGCGCAA CGTTGTTGCC ATTGCTACAG GCATCGTGGT  
 GGTCAATTAT CAAACGCGTT GCAACAACGG TAACGATGTC CGTAGCACC

37751 GTCACGCTCG TCGTTTGGA TGGCTTCATT CAGCTCCGGT TCCCAACGAT  
 CAGTCGCGAGC AGCAAACCAT ACCGAAGTAA GTCGAGGCCA AGGGTTGCTA

37801 CAAGGCGAGT TACATGATCC CCCATGTTGT GCAAAAAAGC GGTTAGCTCC  
 GTTCCGCTCA ATGTACTAGG GGGTACAACA CGTTTTTTCG CCAATCGAGG

37851 TTCGGTCCTC CGATCGTTGT CAGAAGTAAG TTGGCCGCGAG TGTTATCACT  
 AAGCCAGGAG GCTAGCAACA GTCTTCATTC AACCGGCGTC ACAATAGTGA

Figure 26 AN

37901 CATGGTTATG CACTGTC ATAATTCTCT TACTGTCAATG CCATCGTAA  
GTACCAATAC CCGTGACG TATTAAGAGA ATGACAGTAC GGTAGGCTT

37951 GATGCTTTTC TGTGACTGGT GAGTACTCAA CCAAGTCATT CTGAGAATAG  
CTACGAAAAG ACACTGACCA CTCATGAGTT GGTTCAGTAA GACTCTTATC

38001 TGTATGCGGC GACCGAGTTG CTCTTGCCCG GCGTCAACAC GGGATAATAC  
ACATACGCCG CTGGCTCAAC GAGAACGGGC CGCAGTTGTG CCCTATTATG

38051 CGCGCCACAT AGCAGAACTT TAAAAGTGCT CATCATTGGA AAACGTTCTT  
GCGCGGTGTA TCGTCTTGAA ATTTTCACGA GTAGTAACCT TTTGCAAGAA

38101 CGGGGCGAAA ACTCTCAAGG ATCTTACCGC TGTGAGATC CAGTTCGATG  
GCCCCGCTTT TGAGAGTTCC TAGAATGGCG ACAACTCTAG GTCAAGCTAC

38151 TAACCCACTC GTGCACCCAA CTGATCTTCA GCATCTTTTA CTTTCACCAG  
ATTGGGTGAG CACGTGGGTT GACTAGAAGT CGTAGAAAAT GAAAGTGGTC

38201 CGTTTCTGGG TGAGCAAAAA CAGGAAGGCA AAATGCCGCA AAAAAGGGAA  
GCAAAGACCC ACTCGTTTTT GTCCTTCCGT TTTACGGCGT TTTTCCCTT

38251 TAAGGGCGAC ACGGAAATGT TGAATACTCA TACTCTTCCT TTTTCAATAT  
ATTCCCGCTG TGCCTTTACA ACTTATGAGT ATGAGAAGGA AAAAGTTATA

38301 TATTGAAGCA TTTATCAGGG TTATTGTCTC ATGAGCGGAT ACATATTGTA  
ATAACTTCGT AAATAGTCCC AATAACAGAG TACTCGCCTA TGTATAAACT

38351 ATGTATTTAG AAAAATAAAC AAATAGGGGT TCCGCGCACA TTTCCCCGAA  
TACATAAATC TTTTATTTG TTTATCCCCA AGGCGCGTGT AAAGGGGCTT

38401 AAGTGCCACC TGACGTCTAA GAAACCATTA TTATCATGAC ATTAACCTAT  
TTCACGGTGG ACTGCAGATT CTTTGGTAAT AATAGTACTG TAATTGGATA

38451 AAAAATAGGC GTATCACGAG GCCCTTTCGT CTTCAAGAAT TGGATCCGAA  
TTTTTATCCG CATAGTGCTC CGGGAAAGCA GAAGTTCTTA ACCTAGGCTT

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38501 TTCTTAATTT CTTAATTAA (SEQ ID NO:32)  
AAGAATTAAA GAATTAATT (SEQ ID NO:33)

Figure 26 A0

**MRKAd5nef MER1063**  
**(MRKAd5 Pre-Adenoviral Vector Containing the G2A,LLA nef Coding Region)**

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1  CATCATCAAT AATATACCTT ATTTTGGATT GAAGCCAATA TGATAATGAG
   GTAGTAGTTA TTATATGGAA TAAAACCTAA CTTCGGTTAT ACTATTACTC

51  GGGGTGGAGT TTGTGACGTG GCGCGGGGCG TGGGAACGGG GCGGGTGACG
   CCCCACCTCA AACACTGCAC CGCGCCCCGC ACCCTTGCCC CGCCCACTGC

101 TAGTAGTGTG GCGGAAGTGT GATGTTGCAA GTGTGGCGGA ACACATGTAA
   ATCATCACAC CGCCTTCACA CTACAACGTT CACACCGCCT TGTGTACATT

151 GCGACGGATG TGGCAAAAGT GACGTTTTTG GTGTGCGCCG GTGTACACAG
   CGCTGCCTAC ACCGTTTTCA CTGCAAAAC CACACGCGGC CACATGTGTC

201 GAAGTGACAA TTTTCGCGCG GTTTTAGGCG GATGTTGTAG TAAATTGGG
   CTTCACTGTT AAAAGCGCGC CAAAATCCGC CTACAACATC ATTTAAACCC

251 CGTAACCGAG TAAGATTGG CCATTTTCGC GGGAAACTG AATAAGAGGA
   GCATTGGCTC ATTCTAAACC GGTAAAAGCG CCCTTTTGAC TTATTCTCCT

301 AGTGAAATCT GAACAATTTT GTGTTACTCA TAGCGCGTAA TATTTGTCTA
   TCACTTTAGA CTTATTAAAA CACAATGAGT ATCGCGCATT ATAAACAGAT

351 GGGCCCGCGG GACTTTGACC GTTTACGTGG AGACTCGCCC AGGTGTTTTT
   CCGGCGCCCC CTGAAACTGG CAAATGCACC TCTGAGCGGG TCCACAAAAA

401 CTCAGGTGTT TTCCGCGTTC CGGGTCAAAG TTGGCGTTTT ATTATTATAG
   GAGTCCACAA AAGGCGCAAG GCCCAGTTTC AACCGCAAAA TAATAATATC

451 GCGGCGCGCA TCCATTGCAT ACGTTGTATC CATATCATAA TATGTACATT
   CGCCGCGCT AGGTAACGTA TGCAACATAG GTATAGTATT ATACATGTAA

501 TATATTGGCT CATGTCCAAC ATTACCGCCA TGTGACATT GATTATTGAC
   ATATAACCGA GTACAGGTG TAATGGCGGT ACAACTGTAA CTAATAACTG

551 TAGTTATTAA TAGTAATCAA TTACGGGGTC ATTAGTTCAT AGCCCATATA
   ATCAATAATT ATCATTAGTT AATGCCCCAG TAATCAAGTA TCGGGTATAT

601 TGGAGTCCG CGTTACATAA CTTACGGTAA ATGGCCCGCC TGGCTGACCG
   ACCTCAAGGC GCAATGTATT GAATGCCATT TACCGGGCGG ACCGACTGGC

651 CCCAACGACC CCGGCCCATT GACGTCAATA ATGACGTATG TTCCCATAGT
   GGGTTGCTGG GGGCGGGTAA CTGCAGTTAT TACTGCATAC AAGGGTATCA

701 AACGCCAATA GGGACTTTCC ATTGACGTCA ATGGGTGGAG TATTTACGGT
   TTGCGGTTAT CCCTGAAAGG TAACTGCAGT TACCCACCTC ATAAATGCCA

751 AAAGTGCCCA CTTGGCAGTA CATCAAGTGT ATCATATGCC AAGTACGCCC
   TTTGACGGGT GAACCGTCAT GTAGTTCACA TAGTATACGG TTCATGCGGG

801 CCTATTGACG TCAATGACGG TAAATGGCCC GCCTGGCATT ATGCCCCAGTA
   GGATAACTGC AGTTACTGCC ATTTACCGGG CGGACCGTAA TACGGGTCAT

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*Figure 27A*

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851 CATGACCTTA T GACTTTC CTACTTGGCA GTACATCTAC GTATTTA TA
    GTACTGGAAT ACCTGAAAG GATGAACCGT CATGTAGATG CATAATCAGT

901 TCGCTATTAC CATGGTGATG CGGTTTTGGC AGTACATCAA TGGGCGTGGA
    AGCGATAATG GTACCACTAC GCCAAAACCG TCATGTAGTT ACCCGCACCT

951 TAGCGGTTTG ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA
    ATCGCCAAAC TGAGTGCCCC TAAAGGTTCA GAGGTGGGGT AACTGCAGTT

1001 TGGGAGTTTG TTTTGGCACC AAAATCAACG GGACTTTCCA AAATGTCGTA
    ACCCTCAAAC AAAACCGTGG TTTTAGTTGC CCTGAAAGGT TTTACAGCAT

1051 ACAACTCCGC CCCATTGACG CAAATGGGCG GTAGGCGTGT ACGGTGGGAG
    TGTTGAGGCG GGGTAACTGC GTTTACCCGC CATCCGCACA TGCCACCCTC

1101 GTCTATATAA GCAGAGCTCG TTTAGTGAAC CGTCAGATCG CCTGGAGACG
    CAGATATATT CGTCTCGAGC AAATCACTTG GCAGTCTAGC GGACCTCTGC

1151 CCATCCACGC TGTTTTGACC TCCATAGAAG ACACCGGGAC CGATCCAGCC
    GGTAGGTGCG ACAAACTGG AGGTATCTTC TGTGGCCCTG GCTAGGTCGG

1201 TCCGCGGCCG GGAACGGTGC ATTGGAACGC GGATTCCCGG TGCCAAGAGT
    AGCGCGCCGC CCTTGCCACG TAACCTTGCG CTAAGGGGC ACGGTTCTCA

1251 GAGATCTGCC ACCATGGCCG GCAAGTGGTC CAAGAGGTCC GTGCCCCGGT
    CTCTAGACGG TGGTACCGGC CGTTCACCAG GTTCTCCAGG CACGGGCCGA

1301 GGTCCACCGT GAGGGAGAGG ATGAGGAGGG CCGAGCCCGC CGCCGACAGG
    CCAGGTGGCA CTCCCTCTCC TACTCCTCCC GGCTCGGGCG GCGGCTGTCC

1351 GTGAGGAGGA CCGAGCCCGC CGCAGTGGGC GTGGGCGCCG TGTCCAGGGA
    CACTCCTCCT GGCTCGGGCG GCGTCACCCG CACCCGCGGC ACAGGTCCCT

1401 CCTGGAGAAG CACGGCGCCA TCACCTCCTC CAACACCGCC GCCACCAACG
    GGACCTCTTC GTGCCGCGGT AGTGGAGGAG GTTGTGGCGG CGGTGGTTGC

1451 CCGACTGCGC CTGGCTGGAG GCCCAGGAGG ACGAGGAGGT GGGCTTCCCC
    GGCTGACGCG GACCGACCTC CGGGTCTCTC TGCTCCTCCA CCCGAAGGGG

1501 GTGAGGCCCC AGGTGCCCTT GAGGCCCATG ACCTACAAGG GCGCCGTGGA
    CACTCCGGGG TCCACGGGGA CTCCGGGTAC TGGATGTTCC CGCGGCACCT

1551 CCTGTCCCAC TTCCTGAAGG AGAAGGGCGG CCTGGAGGGC CTGATCCACT
    GGACAGGGTG AAGGACTTCC TCTTCCCGCC GGACCTCCCG GACTAGGTGA

1601 CCCAGAAGAG GCAGGACATC CTGGACCTGT GGGTGTACCA CACCCAGGGC
    GGGTCTTCTC CGTCTCTAG GACCTGGACA CCCACATGGT GTGGGTCCCC

1651 TACTTCCCCG ACTGGCAGAA CTACACCCCC GGCCCCGGCA TCAGGTTCCT
    ATGAAGGGGC TGACCGTCTT GATGTGGGGG CCGGGGCCGT AGTCCAAGGG

1701 CCTGACCTTC GGCTGGTGCT TCAAGCTGGT GCCCGTGGAG CCCGAGAAGG
    GGACTGGAAG CCGACCACGA AGTTCGACCA CGGGCACCTC GGGCTCTTCC

1751 TGGAGGAGGC CAACGAGGGC GAGAACAAC TCGCCGCCCA CCCCATGTCC
    ACCTCCTCCG GTTGCTCCCG CTCTTGTTGA CCGGGCGGGT GGGGTACAGG

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Figure 27B



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1801 CAGCACGGCA TGGACCC CGAGAAGGAG GTGCTGGAGT GGAGGTGA
      GTCGTGCCGT AGCTCCTGGG GCTCTTCCTC CACGACCTCA CCTCCAAGCT

1851 CTCCAAGCTG GCCTTCCACC ACGTGGCCAG GGAGCTGCAC CCCGAGTACT
      GAGGTTCGAC CGGAAGGTGG TGCACCGGTC CCTCGACGTG GGGCTCATGA

1901 ACAAGGACTG CTAAAGCCCG GGCAGATCTG CTGTGCCTTC TAGTTGCCAG
      TGTTCCTGAC GATTTCTGGG CCGTCTAGAC GACACGGAAG ATCAACGGTC

1951 CCATCTGTTG TTTGCCCTC CCCCCTGCCT TCCTTGACCC TGAAGGTGC
      GGTAGACAAC AAACGGGGAG GGGGCACGGA AGGAACTGGG ACCTTCCACG

2001 CACTCCCACT GTCCTTTCTT AATAAAATGA GGAAATTGCA TCGCATTGTC
      GTGAGGGTGA CAGGAAAGGA TTATTTTACT CCTTTAACGT AGCGTAACAG

2051 TGAGTAGGTG TCATTCTATT CTGGGGGGTG GGGTGGGGCA GGACAGCAAG
      ACTCATCCAC AGTAAGATAA GACCCCCCAC CCCACCCCGT CCTGTCGTTC

2101 GGGGAGGATT GGAAGACAA TAGCAGGCAT GCTGGGGATG CCGTGGGCTC
      CCCCTCCTAA CCTTCTGTT ATCGTCCGTA CGACCCCTAC GCCACCCGAG

2151 TATGGCCGAT CGCGCGCGCG TACTGAAATG TGTGGGCGTG GCTTAAGGGT
      ATACCGGCTA GCCGCGCGCG ATGACTTTAC ACACCCGCAC CGAATTCCCA

2201 GGGAAAGAAT ATATAAGGTG GGGTCTTAT GTAGTTTGT ATCTGTTTGT
      CCTTTCCTA TATATCCAC CCCAGAATA CATCAAAACA TAGACAAAAC

2251 CAGCAGCCGC CGCGCCCATG AGCACCAACT CGTTTGATGG AAGCATTGTG
      GTCGTCCGCG CGCGCGGTAC TCGTGGTTGA GCAAACCTACC TTCGTAACAC

2301 AGCTCATATT TGACAACGCG CATGCCCCCA TGGGCCGGGG TGCCTCAGAA
      TCGAGTATAA ACTGTTGCGC GTACGGGGGT ACCCGGCCCC ACGCAGTCTT

2351 TGTGATGGGC TCCAGCATTG ATGGTCGCCC CGTCTGCCCC GCAAACCTTA
      ACACIACCCG AGGTCGTAACTACCAGCGGG GCAGGACGGG CGTTTGAGAT

2401 CTACCTTGAC CTACGAGACC GTGTCTGGAA CGCCGTTGGA GACTGCAGCC
      GATGGAAGTG GATGCTCTGG CACAGACCTT GCGGCAACCT CTGACGTCGG

2451 TCCGCCGCCG CTTAGCCGCG TGCAGCCACC GCGCGCGGGA TTGTGACTGA
      AGGCGGCGCG GAAGTCGGCG ACGTCGGTGG CGGGCGCCCT AACACTGACT

2501 CTTTGCTTTC CTGAGCCCGC TTGCAAACAG TGCAGCTTCC CGTTCATCCG
      GAAACGAAAG GACTCGGGCG AACGTTTGTG ACGTCGAAGG GCAAGTAGGC

2551 CCCGCGATGA CAAGTTGACG GCTCTTTTGG CACAATTGGA TTCTTTGACC
      GGGCGCTACT GTTCAACTGC CGAGAAAACC GTGTTAACCT AAGAACTGG

2601 CGGGAACCTA ATGTCGTTTC TCAGCAGCTG TTGGATCTGC GCCAGCAGGT
      GCCCTTGAAT TACAGCAAAG AGTCGTCGAC AACCTAGACG CGGTCGTCCA

2651 TTCTGCCTTG AAGGCTTCCT CCCCTCCCAA TGCGGTTTAA AACATAAATA
      AAGACGGGAC TTCCGAAGGA GGGGAGGGTT ACGCCAAATT TTGTATTTAT

2701 AAAAACCAGA CTCTGTTTGG ATTTGGATCA AGCAAGTGTC TTGCTGTCTT
      TTTTGGTCTT GAGACAAACC TAAACCTAGT TCGTTCACAG AACGACAGAA

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Figure 27C

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2751 TATTTAGGGG TTTTGC GCGGTAGGCC CGGGACCAGC GGTCTCGGTC
    ATAAATCCCC AAAACGCGCG CGCCATCCGG GCCCTGGTCG CCAGAGCCAG

2801 GTTGAGGGTC CTGTGTATTT TTTCCAGGAC GTGGTAAAGG TGA CTCTGGA
    CAACTCCCAG GACACATAAA AAAGTCCTG CACCATTTC ACTGAGACCT

2851 TGTTCAGATA CATGGGCATA AGCCCGTCTC TGGGGTGGAG GTAGCACCAC
    ACAAGTCTAT GTACCCGTAT TCGGGCAGAG ACCCCACCTC CATCGTGGTG

2901 TGCAGAGCTT CATGCTGCGG GGTGGTGTG TAGATGATCC AGTCGTAGCA
    ACGTCTCGAA GTACGACGCC CCACCACAAC ATCTACTAGG TCAGCATCGT

2951 GGAGCGCTGG GCGTGGTGCC TAAAAATGTC TTTCAGTAGC AAGCTGATTG
    CCTCGCGACC CGCACCACGG ATTTTACAG AAAGTCATCG TTCGACTAAC

3001 CCAGGGGCGAG GCCCTTGGTG TAAGTGTTTA CAAAGCGGTT AAGCTGGGAT
    GGTCCCCGTC CGGGAACCAC ATTCACAAAT GTTTCGCCAA TTCGACCCTA

3051 GGGTGCATAC GTGGGATAT GAGATGCATC TTGGACTGTA TTTT TAGGTT
    CCCACGTATG CACCCCTATA CTCTACGTAG AACCTGACAT AAAAATCCAA

3101 GGCTATGTTC CCAGCCATAT CCCTCCGGGG ATTCA GTTG TGCAGAACCA
    CCGATACAAG GGTCCGTATA GGGAGGCCCC TAAGTACAAC ACGTCTTGGT

3151 CCAGCACAGT GTATCCGGTG CACTTGGGAA ATTTGTCTAG TAGCTTAGAA
    GGTCTGTCTA CATAGGCCAC GTGAACCCCT TAAACAGTAC ATCGAATCTT

3201 GGAAATGCGT GGAAGAACTT GGAGACGCCC TTGTGACCTC CAAGATTTTC
    CCTTTACGCA CCTTCTTGAA CCTCTCGGG AACACTGGAG GTTCTAAAAG

3251 CATGCATTCTG TCCATAATGA TGGCAATGGG CCCACGGGCG GCGGCCTGGG
    GTACGTAAGC AGGTATTACT ACCGTTACCC GGGTGCCCGC CGCCGGACCC

3301 CGAAGATATT TCTGGGATCA CTAACGTCAT AGTTGTGTTT CAGGATGAGA
    GCTTCTATAA AGACCCTAGT GATTGCAGTA TCAACACAAG GTCCTACTCT

3351 TCGTCATAGG CCATTTTAC AAAGCGCGGG CGGAGGGTGC CAGACTGCGG
    AGCAGTATCC GGTAAAATG TTTCCGCGCC GCCTCCACG GTCTGACGCC

3401 TATAATGGTT CCATCCGGCC CAGGGGCGTA GTTACCTCA CAGATTTGCA
    ATATTACCAA GGTAGGCCGG GTCCCCGCAT CAATGGGAGT GTCTAAACGT

3451 TTTCCACGCG TTTGAGTTCA GATGGGGGA TCATGTCTAC CTGCGGGGCG
    AAAGGGTGCG AAAC TCAAGT CTACCCCT AGTACAGATG GACGCCCCGC

3501 ATGAAGAAAA CGGTTTCCGG GTAGGGGAG ATCAGCTGGG AAGAAAGCAG
    TACTTCTTTT GCCAAAGGCC CCATCCCTC TAGTCGACCC TTCTTTCGTC

3551 GTTCTTGAGC AGCTGCGACT TACCGCAGCC GGTGGGCCCC TAAATCACAC
    CAAGGACTCG TCGACGCTGA ATGGCGTCGG CCACCCGGGC ATTTAGTGTG

3601 CTATTACCGG CTGCAACTGG TAGTTAAGAG AGCTGCAGCT GCCGTATCC
    GATAATGGCC GACGTTGACC ATCAATTCTC TCGACGTCGA CGGCAGTAGG

3651 CTGAGCAGGG GGGCCACTTC GTTAAGCATG TCCCTGACTC GCATGTTTTC
    GACTCGTCCC CCCGGTGAAG CAATTCGTAC AGGGACTGAG CGTACAAAAG

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Figure 27D

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3701 CCTGACCAAA BCCAGAA GCGCTCGCC GCCAGCGAT AGCAGT T
    GCGCTGTTT AGGCGGTCTT CCGCGAGCGG CGGGTCGCTA TCGTCAAGAA

3751 GCAAGGAAGC AAAGTTTTTC AACGGTTTGA GACCGTCCGC CGTAGGCATG
    CGTTCCTTCG TTTCAAAAAG TTGCCAAACT CTGGCAGGCG GCATCCGTAC

3801 CTTTTGAGCG TTTGACCAAG CAGTTCCAGG CGGTCCCACA GCTCGGTCAC
    GAAACTCGC AACTTGTTTC GTCAAGGTCC GCCAGGGTGT CGAGCCAGTG

3851 CTGCTCTACG GCATCTCGAT CCAGCATATC TCCTCGTTTC GCGGGTTGGG
    GACGAGATGC CGTAGAGCTA GGTCGTATAG AGGAGCAAAG CGCCCAACCC

3901 GCGGCTTTTCG CTGTACGGCA GTAGTCGGTG CTCGTCCAGA CCGGCCAGGG
    CGCCGAAAGC GACATGCCGT CATCAGCCAC GAGCAGGTCT GCCCGTCCC

3951 TCATGTCTTT CCACGGGCGC AGGGTCCTCG TCAGCGTAGT CTGGGTCACG
    AGTACAGAAA GGTGCCCGCG TCCCAGGAGC AGTCGCATCA GACCCAGTGC

4001 GTGAAGGGGT GCGCTCCGGG CTGCGCGCTG GCCAGGGTGC GCTTGAGGCT
    CACTTCCCCA CCGGAGGCCG GACGCGCGAC CGGTCCCACG CGAACTCCGA

4051 GGTCTCTGTC GTGCTGAAGC GCTGCCGGTC TTCGCCCTGC GCGTCGGCCA
    CCAGGACGAC CACGACTTCG CGACGGCCAG AAGCGGGACG CGCAGCCGGT

4101 GGTAGCATTT GACCATGGTG TCATAGTCCA GCGGCTCCGC GGCGTGCGCC
    CCATCGTAAA CTGGTACCAC AGTATCAGGT CCGGGAGGCG CCGCACCGGG

4151 TTGGCGCGCA GCTTGCCCTT GGAGGAGGCG CCGCACGAGG GGCAGTGCAG
    AACCGCGCGT CGAACGGGAA CCTCCTCCGC GCGGTGCTCC CCGTCACGTC

4201 ACTTTTGAGG GCGTAGAGCT TGGGCGCGAG AAATACCGAT TCCGGGGAGT
    TGAAACTCC CGCATCTCGA ACCCGCGCTC TTTATGGCTA AGGCCCCTCA

4251 AGGCATCCGC GCCGCAGGCC CCGCAGACGG TCTCGCATTC CACGAGCCAG
    TCCGTAGGCG CCGCGTCCGG GCGGTCTGCC AGAGCGTAAG GTGCTCGGTC

4301 GTGAGCTCTG GCCGTTCCGG GTCAAAAACC AGGTTTCCCC CATGCTTTTT
    CACTCGAGAC CGGCAAGCCC CAGTTTTTGG TCCAAAGGGG GTACGAAAAA

4351 GATGCGTTTC TTACCTCTGG TTTCCATGAG CCGGTGTCCA CGCTCGGTGA
    CTACGCAAAG AATGGAGACC AAAGGTACTC GGCCACAGGT GCGAGCCACT

4401 CGAAAAGGCT GTCCGTGTCC CCGTATACAG ACTTGAGAGG CCTGTCTCTG
    GCTTTTCCGA CAGGCACAGG GGCATATGTC TGAATCTCC GGACAGGAGC

4451 AGCGGTGTTTC CGCGTCTCTC CTCGTATAGA AACTCGGACC ACTCTGAGAC
    TCGCCACAAG GCGCCAGGAG GAGCATATCT TTGAGCCTGG TGAGACTCTG

4501 AAAGGCTCGC GTCCAGGCCA GCACGAAGGA GGCTAAGTGG GAGGGGTAGC
    TTTCCGAGCG CAGGTCCGGT CGTGCTTCCT CCGATTACCC CTCCCCATCG

4551 GGTGCTGTGC CACTAGGGGG TCCACTCGCT CCAGGGTGTG AAGACACATG
    CCAGCAACAG GTGATCCCCC AGGTGAGCGA GGTCCACAC TTCTGTGTAC

4601 TCGCCCTCTT CCGCATCAAG GAAGGTGATT GGTGTGTAGG TGTAGGCCAC
    AGCGGGAGAA GCCGTAGTTC CTTCCACTAA CCAAACATCC ACATCCGGTG

```

Figure 27E

4651 GTGACCGGGT CCTGAAG GGGGGCTATA AAAGGGGGTG GGGGCCCTT  
 CACTGGCCCA CAAGGACTTC CCCCCGATAT TTTCCCCCAC CCCC GC GCAA  
 4701 CGTCCTCACT CTCTTCCGCA TCGCTGTCTG CGAGGGCCAG CTGTTGGGGT  
 GCAGGAGTGA GAGAAGGCGT AGCGACAGAC GCTCCCGGTC GACAACCCCA  
 4751 GAGTACTCCC TCTGAAAAGC GGGCATGACT TCTGCGCTAA GATTGTCAGT  
 CTCATGAGGG AGACTTTTCG CCCGTACTGA AGACGCGATT CTAACAGTCA  
 4801 TTCCAAAAAC GAGGAGGATT TGATATTAC CTGGCCCCGC GTGATGCCTT  
 AAGGTTTTTG CTCTCCTAA ACTATAAGTG GACCGGGCGC CACTACGGAA  
 4851 TGAGGGTGGC CGCATCCATC TGGTCAGAAA AGACAATCTT TTTGTTGTCA  
 ACTCCACCG GCGTAGGTAG ACCAGTCTTT TCTGTTAGAA AAACAACAGT  
 4901 AGCTTGGTGG CAAACGACCC GTAGAGGGCG TTGGACAGCA ACTTGGCGAT  
 TCGAACCACC GTTTGCTGGG CATCTCCGC AACCTGTCGT TGAACCGCTA  
 4951 GGAGCGCAGG GTTTGGTTTT TGTGCGGATC GCGCGCTCC TTGGCCGCGA  
 CCTCGCTCC CAAACCAAAA ACAGCGCTAG CCGCGCAGG AACCGGCGCT  
 5001 TGTTTAGCTG CACGTATTG CCGCAACGC ACCGCCATTC GGGAAAGACG  
 ACAAATCGAC GTGCATAAGC GCGCGTTGCG TGGCGGTAAG CCTTTCTGC  
 5051 GTGGTGCGCT CGTCGGGCAC CAGGTGCACG CGCCAACCGC GGTGTGTCAG  
 CACCACGCGA GCAGCCCGTG GTCCACGTGC GCGGTTGGCG CCAACACGTC  
 5101 GGTGACAAGG TCAACGCTGG TGGCTACCTC TCCGCGTAGG CGCTCGTTGG  
 CCACGTGTTCC AGTTGCGACC ACCGATGGAG AGGCGCATCC GCGAGCAACC  
 5151 TCCAGCAGAG GCGGCCGCC TTGCGCGAGC AGAATGGCGG TAGGGGTCT  
 AGGTGCTCTC CGCCGCGGGG AACGCGCTCG TCTTACCGCC ATCCCCAGA  
 5201 AGCTGCGTCT CGTCCGGGGG GTCTGCGTCC ACGGTAAAGA CCCCGGGCAG  
 TCGACGCGA GCAGGCCCCC CAGACGAGG TGCCATTCTT GGGGCCCGTC  
 5251 CAGGCGCGCG TCGAAGTAGT CTATCTTGCA TCCTTGCAAG TCTAGCGCCT  
 GTCCGCGCGC AGCTTCATCA GATAGAACGT AGGAACGTTC AGATCGCGGA  
 5301 GCTGCCATGC GCGGCGGCA AGCGCGCGCT CGTATGGGTT GAGTGGGGGA  
 CGACGGTACG GCGCCGCCGT TCGCGCGCGA GCATACCCAA CTCACCCCT  
 5351 CCCCATGGCA TGGGGTGGGT GAGCGCGGAG GCGTACATGC CGCAAATGTC  
 GGGGTACCGT ACCCCACCCA CTCGCGCCTC CGCATGTACG GCGTTTACAG  
 5401 GTAAACGTAG AGGGGCTCTC TGAGTATTCC AAGATATGTA GGSTAGCATC  
 CATTTGCATC TCCCCGAGAG ACTCATAAGG TTCTATACAT CCCATCGTAG  
 5451 TTCCACCGCG GATGCTGGCG CGCACGTAAT CGTATAGTTC GTGCGAGGGA  
 AAGGTGGCGC CTACGACCGC GCGTGCAATTA GCATATCAAG CACGCTCCCT  
 5501 GCGAGGAGGT CGGGACCGAG GTTGCTACGG GCGGGCTGCT CTGCTCGGAA  
 CGCTCCTCCA GCGCTGGCTC CAACGATGCC CGCCCGACGA GACGAGCCTT  
 5551 GACTATCTGC CTGAAGATGG CATGTGAGTT GGATGATATG GTTGACGCT  
 CTGATAGACG GACTTCTACC GTACACTCAA CCTACTATAC CAACCTGCGA

Figure 27F

5601 GGAAGACGTT CTTCTGGCG TCTGTGAGAC CTACCGCGTC ACGCACTG  
CCTTCTGCAA CTTGACCGC AGACACTCTG GATGGCGCAG TCGGTGCTTC

5651 GAGGCGTAGG AGTCGCGCAG CTTGTTGACC AGCTCGGCGG TGACCTGCAC  
CTCCGCATCC TCAGCGCGTC GAACAACTGG TCGAGCCGCC ACTGGACGTG

5701 GTCTAGGGCG CAGTAGTCCA GGGTTTCCTT GATGATGTCA TACTTATCCT  
CAGATCCCGC GTCATCAGGT CCCAAAGGAA CTACTACAGT ATGAATAGGA

5751 GTCCCTTTTT TTTCCACAGC TCGCGGTTGA GGACAACTC TTGCGGTCT  
CAGGGAAAAA AAAGGTGTCG AGCGCCAAC TCTGTTGAG AAGCGCCAGA

5801 TTCCAGTACT CTTGGATCGG AAACCCGTCG GCCTCCGAAC GGTAAGAGCC  
AAGGTCATGA GAACCTAGCC TTTGGGCAGC CGGAGGCTTG CCATTCTCGG

5851 TAGCATGTAG AACTGGTTGA CGGCCTGGTA GGCGCAGCAT CCCTTTTCTA  
ATCGTACATC TTGACCAACT GCCGGACCAT CCGCGTCGTA GGGAAAAGAT

5901 CCGGTAGCGC GTATGCCTGC GCGGCTTCC GGAGCGAGGT GTGGGTGAGC  
GCCCATCGCG CATACGGACG CGCCGGAAGG CCTCGCTCCA CACCCACTCG

5951 GCAAAGGTGT CCCTGACCAT GACTTTGAGG TACTGGTATT TGAAGTCAGT  
CSTTTCCACA GGGACTGGTA CTGAACTCC ATGACCATAA ACTTCAGTCA

6001 GTCGTCCGAT CCGCCCTGCT CCCAGAGCAA AAAGTCCGTG CGCTTTTGG  
CAGCAGCGTA GCGGGGACGA GGGTCTCGTT TTTCAGGCAC GCGAAAAACC

6051 AACGCGGATT TGGCAGGGCG AAGGTGACAT CGTTGAAGAG TATCTTTCCC  
TTGCGCCTAA ACCGTCCCGC TTCCACTGTA GCAACTTCTC ATAGAAAGGG

6101 GCGCGAGGCA TAAAGTTGCG TGTGATGCGG AAGGGTCCC GACCTCGGA  
CGCGCTCCGT ATTCAACGC AACTACGCC TTCCAGGGC CGTGGAGCCT

6151 ACGGTTGTA ATTACCTGGG CGGCGAGCAC GATCTCGTCA AAGCCGTTGA  
TGCCAACAAT TAATGGACCC GCGCTCGTG CTAGAGCAGT TTCGGCAACT

6201 TGTGTGGCC CACAAATGTAA AGTTCCAAGA AGCGCGGGAT GCCCTTGATG  
ACAACACCGG GTGTACATT TCAAGTTCT TCGCGCCCTA CGGGAACCTAC

6251 GAAGGCAATT TTTTAAGTTC CTCGTAGGTG AGCTCTTCAG GGGAGCTGAG  
CTTCGTTAA AAAATTCAAG GAGCATCCAC TCGAGAAGTC CCCTCGACTC

6301 CCCGTGCTCT GAAAGGGCCC AGTCTGCAAG ATGAGGGTTG GAAGCGACGA  
GGGCACGAGA CTTTCCCGGG TCAGACGTTT TACTCCCAAC CTTGCTGCT

6351 ATGAGCTCCA CAGGTCACGG GCCATTAGCA TTTGCAGGTG GTCGCGAAAG  
TACTCGAGGT GTCCAGTGCC CGGTAATCGT AAACGTCCAC CAGCGCTTTC

6401 GTCCTAACT GCGACCTAT GGCCATTTTT TCTGGGGTGA TGCAGTAGAA  
CAGGATTTGA CCGCTGGATA CCGGTAAAAA AGACCCCACT ACGTCATCTT

6451 GGTAAGCGGG TCTTGTTCCC AGCGGTCCCA TCCAAGGTTT GCGGCTAGGT  
CCATTGCGCC AGAACAAGGG TCGCCAGGCT AGGTTCCAAG CGCCGATCCA

6501 CTCGCGCGGC AGTCACTAGA GGCTCATCTC CGCCGAACCT CATGACCAGC  
GAGCGCGCCG TCAGTGATCT CCGAGTAGAG GCGGCTTGAA GTACTGGTCG

Figure 27G

6551 ATGAAGGGCA ( ) CTGCTT CCCAAAGGCC CCCATCCAAG TATAGG ( ) TC  
 TACTTCCCGT GCTCGACGAA GGGTTTCCGG GGGTAGGTTC ATATCCAGAG  
 6601 TACATCGTAG GTGACAAAGA GACGCTCGGT GCGAGGATGC GAGCCGATCG  
 ATGTAGCATC CACTGTTTCT CTGCGAGCCA CGCTCCTACG CTCGGCTAGC  
 6651 GGAAGAACTG GATCTCCCGC CACCAATTGG AGGAGTGGCT ATTGATGTGG  
 CCTTCTTGAC CTAGAGGGCG GTGGTTAACC TCCTCACC GA TAACTACACC  
 6701 TGAAAGTAGA AGTCCCTGCG ACGGGCCGAA CACTCGTGCT GGCTTTTGTA  
 ACTTTTCATCT TCAGGACGCG TGCCCGGCTT GTGAGCACGA CCGAAAACAT  
 6751 AAAACGTGCG CAGTACTGGC AGCGGTGCAC GGGCTGTACA TCCTGCACGA  
 TTTTGACACG GTCATGACCG TCGCCACGTG CCCGACATGT AGGACGTGCT  
 6801 GGTGACCTG ACGACCGCGC ACAAGGAAGC AGAGTGGGAA TTTGAGCCCC  
 CCAACTGGAC TGCTGCGCGG TGTTCCTTCG TCTACCCCTT AACTCGGGG  
 6851 TCGCCTGGCG GGTTCGGCTG GTGGTCTTCT ACTTCGGCTG CTTGTCCTTG  
 AGCGGACCGC CCAAACCGAC CACCAGAAGA TGAAGCCGAC GAACAGGAAC  
 6901 ACCGTC TGCTCGAGGG GAGTTACGGT GGATCGGACC ACCACGCCGC  
 TGCGAGACCG ACGAGCTCCC CTCAATGCCA CCTAGCCTCG TGGTGCGGCG  
 6951 GCGAGCCCAA AGTCCAGATG TCCGCGCGCG GCGGTCCGAG CTTGATGACA  
 CGCTCGGGTT TCAGGTCTAC AGGCGCGCGC CGCCAGCCTC GAACTACTGT  
 7001 ACATCGCGCA GATGGGAGCT GTCCATGGTC TGGAGCTCCC GCGGCGTCAG  
 TGTAGCGCGT CTACCCTCGA CAGGTACCAG ACCTCGAGGG CGCCGCAGTC  
 7051 GTCAGGCGGG AGCTCCTGCA GGTTCCTTC GCATAGACGG GTCAGGGCGC  
 CAGTCCGCCC TCGAGGACGT CCAAATGGAG CGTATCTGCC CAGTCCGCG  
 7101 GGGCTAGATC CAGGTGATAC CTAATTCCA GGGGCTGGTT GGTGGCGGCG  
 CCCGATCTAG GTCCACTATG GATTAAAGGT CCCCAGCAA CCACCGCCGC  
 7151 TCGATGGCTT GCAAGAGGCC GCATCCCCGC GCGCGACTA CGGTACCGCG  
 AGCTACCGAA CGTTCTCCGG CGTAGGGGCG CCGCGCTGAT GCCATGGCGC  
 7201 CGCGGGGCGG TGGGCGCGCG GGGTGTCTT GGATGATGCA TCTAAAAGCG  
 GCCGCCGCC ACCCGGCGCC CCCACAGGAA CCTACTACGT AGATTTTCGC  
 7251 GTGACGCGGG CGAGCCCCCG GAGGTAGGGG GGGCTCCGGA CCCGCCGGGA  
 CACTGCGCCC GCTCGGGGGC CTCCATCCCC CCCGAGGCTT GGGCGGCCCT  
 7301 GAGGGGGCAG GGGCACGTCG GCGCCGCGCG GGGCAGGAG CTGGTGCTGC  
 CTCCCCGTC CCGTGACAGC CGCGCGCGCG GCGGTCCTC GACCACGACG  
 7351 GCGCGTAGGT TGCTGGCGAA CGCGACGACG GGGCGGTTGA TCTCCTGAAT  
 CGCGCATCCA ACGACCGCTT GCGCTGCTGC GCCGCCAACT AGAGGACTTA  
 7401 CTGGCGCCTC TGGTGAAGA CGACGGGCCC GGTGAGCTTG AACCTGAAAG  
 GACCGCGGAG ACGCACTTCT GCTGCCCCGG CCACTCGAAC TTGGACTTTC  
 7451 AGAGTTCGAC AGAATCAATT TCGGTGTCTG TGACGGCGGC CTGGCGCAAA  
 TCTCAAGCTG TCTTAGTTAA AGCCACAGCA ACTGCCGCGG GACCGCGTTT

Figure 27H

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7501 ATCTCCTGCA CACTCCTGGA GTTGTCTTGA TAGGCGATGT GGGCEATGAA
TAGAGGACGT GAGGAGGACT CAACAGAACT ATCCGCTAGA GCCGGTCTT

7551 CTGCTCGATC TCTTCCTCCT GGAGATCTCC GCGTCCGGCT CGCTCCACGG
GACGAGCTAG AGAAGGAGGA CCTCTAGAGG CGCAGGCCGA GCGAGGTGCC

7601 TGGCGGCGAG GTCGTGGAAT ATGCGGGCCA TGAGCTGCGA GAAGGCGTTG
ACCGCCGCTC CAGCAACCTT TACGCCCGGT ACTCGACGCT CTTCCGCAAC

7651 AGGCCTCCCT CGTTCAGAC GCGGCTGTAG ACCACGCCCC CTTCCGGCATC
TCCGGAGGGA GCAAGGTCTG CGCCGACATC TGGTCCGGGG GAAGCCGTAG

7701 GCGGGCGCGC ATGACCACCT GCGCGAGATT GAGCTCCACG TGCCGGGCGA
CGCCCGCGCG TACTGGTGGA CGCGCTCTAA CTCGAGGTGC ACGGCCCGCT

7751 AGACGGCGTA GTTTCGCGAG CGCTGAAAGA GGTAAGTTGAG GGTGGTGGCG
TCTGCCGCAT CAAAGCGTCC GCGACTTTCT CCATCAACTC CCACCACCGC

7801 GTGTGTCTCT CCACGAAGAA GTACATAACC CAGCGTCGCA ACGTGGATTC
CACACAAGAC GGTGCTTCTT CATGTATTGG GTCGCGAGCGT TGCACCTAAG

7851 GTTGATATCC CCCAAGGCCT CAAGCGCTC CATGGCCTCG TAGAAGTCCA
CAACTATAGG GGGTTCGGGA GTTCCGCGAG GTACCGGAGC ATCTTCAGGT

7901 CGGCGAAGTT GAAAACTGG GAGTTGCGCG CCGACACGGT TAACTCCTCC
GCCGCTTCAA CTTTTGTACC CTCAACGCGC GGCTGTGCCA ATTGAGGAGG

7951 TCCAGAAGAC GGATGAGCTC GGCACAGTG TCGCGCACCT CGCGCTCAAA
AGGTCTTCTG CCTACTCGAG CCGCTGTCAC AGCGCGTGGA GCGCGAGTTT

8001 GGCTACAGGG GCCTCTTCTT CTTCTTCAAT CTCTCTTCC ATAAGGGCCT
CCGATGTCCC CGGAGAAGAA GAAGAAGTTA GAGGAGAAGG TATTCCCGGA

8051 CCCCTTCTTC TTCTTCTGGC GCGGTTGGGG GAGGGGGGAC ACGGCGGCGA
GGGAAGAAG AAGAAGACCG CCGCCACCCC CTCCCCCTG TGCCGCCGCT

8101 CGACGGCGCA CCGGGAGGCG GTCGACAAAG CGCTCGATCA TCTCCCCGCG
GCTGCCGCGT GGCCCTCCGC CAGCTGTTTC GCGAGCTAGT AGAGGGGCGC

8151 GCGACGGCGC ATGGTCTCGG TGACGGCGCG GCCGTCTCTG CGGGGGCGCA
CGCTGCCGCG TACCAGAGCC ACTGCCGCGC CGGCAAGAGC GCCCCCGCGT

8201 GTTGGAAGAC GCCGCCCGTC ATGTCCCGGT TATGGGTTGG CGGGGGGCTG
CAACCTTCTG CGGCGGGCAG TACAGGGCCA ATACCCAACC GCCCCCGAC

8251 CCATGCGGCA GGGATACGGC GCTAACGATG CATCTCAACA ATTGTTGTGT
GGTACGCCGT CCTATGCCG CGATTGCTAC GTAGAGTTGT TAACAACACA

8301 AGGTACTCCG CCGCCGAGGG ACCTGAGCGA GTCCGCATCG ACCGGATCGG
TCCATGAGGC GCGGGCTCCC TGGACTCGCT CAGGCGTAGC TGGCCTAGCC

8351 AAAACCTCTC GAGAAAGGCG TCTAACCACT CACAGTCGCA AGGTAGGCTG
TTTTGGAGAG CTCTTTCGCG AGATTGGTCA GTGTCAGCGT TCCATCCGAC

8401 AGCACCGTGG CGGCGGGCAG CGGGCGGCGG TCGGGGTTGT TTCTGGCGGA
TCGTGGCACC GCGCGCCGTC GCGCGCGGCC AGCCCCAACA AAGACCGCCT

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Figure 27I

8451 GGTGCTGCTG ~~AT~~GTAAAT TAAAGTAGGC GGTCTTGAGA CGGCGG~~EG~~  
 CCACGACGAC TATACATTA ATTTTCATCCG CCAGAACTCT GCGCGCTACC  
 8501 TCGACAGAAG CACCATGTCC TTGGGTCCGG CCTGCTGAAT GCGCAGGCGG  
 AGCTGTCTTC GTGGTACAGG AATCCAGGCC GGACGACTTA CCGTCCGCGC  
 8551 TCGGCCATGC CCCAGGCTTC GTTTTGACAT CGGCGCAGGT CTTGTAGTA  
 AGCCGGTACG GGGTCCGAAG CAAAACGTGA GCCGCGTCCA GAAACATCAT  
 8601 GTCTTGCAATG AGCCTTCTTA CCGGCACCTC TTCTTCTCCT TCCTCTTGTC  
 CAGAACGTAC TCGGAAAGAT GGCCGTGAAG AAGAAGAGGA AGGAGAACAG  
 8651 CTGCATCTCT TGCATCTATC GCTGCGGCGG CGGCGGAGTT TGGCCGTAGG  
 GACGTAGAGA ACGTAGATAG CGACGCCGCC GCCGCTCAA ACCGGCATCC  
 8701 TGGCGCCCTC TTCCTCCCAT GCGTGTGACC CCGAAGCCCC TCATCGGCTG  
 ACCCGGGGAG AAGGAGGGTA CGCACACTGG GGCTTCGGGG AGTAGCCGAC  
 8751 AAGCAGGGCT AGGTCCGCCA CAACGCGCTC GGCTAATATG GCCTGCTGCA  
 TTCGTCCCGA TCCAGCCGCT GTTGCGCGAG CCGATTATAC CGGACGACGT  
 8801 CCTGCGTGAG GGTAGACTGG AAGTCATCCA TGTCCACAAA GCGGTGGTAT  
 GGACGCACTC CCATCTGACC TTCAGTAGGT ACAGGTGTTT CGCCACCATA  
 8851 GCGCCCGTGT TGATGGTGTA AGTGCACTTG GCCATAACGG ACCAGTTAAC  
 CGCGGGCACA ACTACCACAT TCACGTCAAC CGGTATTGCC TGGTCAATTG  
 8901 GGTCTGGTGA CCCGCTGCG AGAGCTCGGT GTACCTGAGA CGCGAGTAAG  
 CCAGACCACT GGGCCGACGC TCTCGAGCCA CATGGACTCT GCGCTCATTC  
 8951 CCCTCGAGTC AAATACGTAG TCGTTGCAAG TCCGCACCAG GTACTGGTAT  
 GGGAGCTCAG TTTATGCATC AGCAACGTTT AGGCGTGGTC CATGACCATA  
 9001 CCCACCAAAA AGTGCGGCGG CGGCTGGCGG TAGAGGGGCC AGCGTAGGGT  
 GGGTGGTTTT TCACGCCGCC GCCGACCGCC ATCTCCCCGG TCGCATCCCA  
 9051 GGGCGGGGCT CCGGGGGCGA GATCTTCCAA CATAAGGCGA TGATATCCGT  
 CCGGCCCCGA GGCCCCGCT CTAGAAGGTT GTATTCCGCT ACTATAGGCA  
 9101 AGATGTACCT GGACATCCAG GTGATGCCGG CGGCGGTGGT GGAGGCGCGC  
 TCTACATGGA CTTGTAGGTC CACTACGGCC GCCGCCACCA CCTCCGCGCG  
 9151 GGAAAGTCGC GGACGCGGTT CCAGATGTTG CGCAGCGGCA AAAAGTGCTC  
 CCTTTCAGCG CCTGCGCCAA GGTCTACAAC GCGTCGCCGT TTTTCACGAG  
 9201 CATGGTCGGG ACGCTCTGGC CGGTCAGGCG CGCGCAATCG TTGACGCTCT  
 GTACCAGCCC TGCAGAGCCG GCCAGTCCGC GCGCGTTAGC AACTGCGAGA  
 9251 AGACCGTGCA AAAGGAGAGC CTGTAAGCGG GCACTCTTCC GTGGTCTGGT  
 TCTGGCACGT TTTCTCTCG GACATTGCGC CGTGAGAAGG CACCAGACCA  
 9301 GGATAAATTC GCAAGGGTAT CATGGCGGAC GACCGGGGTT CGAGCCCCGT  
 CCTATTTAAG CGTCCCATTA GTACCGCCTG CTGGCCCCAA GCTCGGGGCA  
 9351 ATCCGGCCGT CCGCCGTGAT CCATGCGGTT ACCGCCCGCG TGTGAAACCC  
 TAGGCCGGCA GGCGGCACTA GGTACGCCAA TGGCGGCGC ACAGCTTGGG

Figure 27J



9401 AGGTGTGCGA GAGACAA CGGGGGAGTG CTCCTTTTGG CTTCCCTTAA  
 TCCACACGCT GCAGTCTGTT GCCCCCTCAC GAGGAAAACC GAAGGAAGGT  
 9451 GGCGCGGCGG CTGCTGCGCT AGCTTTTTTG GCCACTGGCC GCGCGCAGCG  
 CCGCGCCGCC GACGACGCGA TCGAAAAAAC CGGTGACCGG GCGCGCTCGC  
 9501 TAAGCGGTTA GGCTGGAAG CGAAAGCATT AAGTGGCTCG CTCCCTGTAG  
 ATTCGCCAAT CCGACCTTTC GCTTTCGTAA TTCACCGAGC GAGGGACATC  
 9551 CCGGAGGGTT ATTTTCCAAG GGTGAGTCG CGGGACCCCC GGTTCGAGTC  
 GGCCTCCCAA TAAAAGGTTT CCAACTCAGC GCCCTGGGGG CCAAGCTCAG  
 9601 TCGGACCGGC CGGACTGCGG CGAACGGGGG TTTGCCTCCC CGTCATGCAA  
 AGCCTGGCCG GCCTGACGCC GCTTGCCCCC AAACGGAGGG GCAGTACGTT  
 9651 GACCCCGCTT GCAAATTCCT CCGGAAACAG GGACGAGCCC CTTTTTTGCT  
 CTGGGGCGAA CGTTTAAGGA GGCCTTTGTC CCTGCTCGGG GAAAAAACGA  
 9701 TTTCCAGAT GCATCCGGTG CTGCGGCAGA TGCGCCCCC TCCTCAGCAG  
 AAAGGGCTA CGTAGGCCAC GACGCCGTCT ACGCGGGGGG AGGAGTCGTC  
 9751 CGGCAAGAGC AAGAGCAGCG GCAGACATGC AGGGCACCTT CCCCTCCTCC  
 GCCGTTCCTG TTCTCGTCGC CGTCTGTACG TCCCGTGGGA GGGGAGGAGG  
 9801 TACCGCGTCA GGAGGGCGCA CATCCGCGGT TGACGCGGCA GCAGATGGTG  
 ATGGCGCAGT CTTCCCGCT GTAGGCGCCA ACTGCGCGGT CGTCTACCAC  
 9851 ATTACGAACC CCCGCGGCGC CGGGCCCGGC ACTACCTGGA CTTGGAGGAG  
 TAATGCTTGG GGGCGCCGCG GCCCGGGCCG TGATGGACCT GAACCTCCTC  
 9901 GGCGAGGGCC TGGCGCGGCT AGGAGCGCCC TCTCTGAGC GGCACCCAAG  
 CCGCTCCCGG ACCGCGCCGA TCCTCGCGGG AGAGGACTCG CCGTGGGTTT  
 9951 GGTGCAGCTG AAGCGTGATA CGCGTGAGGC GTACGTGCCG CGGCAGAACG  
 CCACGTGAC TTGCACTAT GCGCACTCCG CATGCACGGC GCCGTCTTGG  
 10001 TGTTCGCGA CCGCGAGGGA GAGGAGCCCG AGGAGATGCG GGATCGAAAG  
 ACAAAGCGCT GCGCTCCCT CTCTCGGGC TCCTCTACGC CCTAGCTTTC  
 10051 TTCCACGCAG GCGCGAGCT GCGGCATGGC CTGAATCGCG AGCGGTGCT  
 AAGGTGCGTC CCGCGCTCGA CGCCGTACCG GACTTAGCGC TCGCCAACGA  
 10101 GCGCGAGGAG GACTTTGAGC CCGACGCGCG AACCGGATT AGTCCCGCGC  
 CGCGCTCCTC CTGAAACTCG GGCTGCGCGC TTGGCCCTAA TCAGGGCGCG  
 10151 GCGCACACGT GCGGGCCGCC GACCTGGTAA CCGCATACGA GCAGACGGTG  
 CCGGTGTGCA CCGCCGGCGG CTGGACCATT GGCCTATGCT CGTCTGCCAC  
 10201 AACCAGGAGA TTAACCTTCA AAAAAGCTTT AACAACCAGG TCGGTACGCT  
 TTGGTCTCT AATTGAAAGT TTTTTCGAAA TTGTTGGTGC ACGCATGCGA  
 10251 TGTGGCGCGC GAGGAGGTGG CTATAGGACT GATGCATCTG TGGGACTTTG  
 ACACCGCGCG CTCCTCCACC GATATCCTGA CTACGTAGAC ACCCTGAAAC  
 10301 TAAGCGCGCT GGAGCAAAAC CCAAATAGCA AGCCGCTCAT GCGCGAGCTG  
 ATTCGCGCGA CTCGTTTTG GGTATTATCGT TCGGCGAGTA CCGCGTCGAC

Figure 27K

10351 TTCCTTATAG TGCACAG CAGGGACAAC GAGGCATTCA GGGATG T  
 AAGGAATATC ACCTCGTGTC GTCCCTGTTG CTCCGTAAGT CCTTACGGA  
 10401 GCTAAACATA GTAGAGCCCG AGGGCCGCTG GCTGCTCGAT TTGATAAACA  
 CGATTTGTAT CATCTCGGGC TCCCGCGGAC CGACGAGCTA AACTATTTGT  
 10451 TCCTGCAGAG CATAGTGGTG CAGGAGCGCA GCTTGAGCCT GGCTGACAAG  
 AGGACGTCTC GTATCACCAC GTCCTCGCGT CGAACTCGGA CCGACTGTTC  
 10501 GTGGCCGCCA TCAACTATTC CATGCTTAGC CTGGGCAAGT TTTACGCCCC  
 CACCGGCGGT AGTTGATAAG GTACGAATCG GACCCGTTCA AAATGCGGGC  
 10551 CAAGATATAC CATACCCCTT ACCTTCCCAT AGACAAGGAG GTAAAGATCG  
 GTTCTATATG GTATGGGGAA TGCAAGGGTA TCTGTTCTCT CATTTCTAGC  
 10601 AGGGGTTCTA CATGCGCATG GCGCTGAAGG TGCTTACCTT GAGCGACGAC  
 TCCCCAAGAT GTACGCGTAC CGCGACTTCC ACGAATGGAA CTCGCTGCTG  
 10651 CTGGGCGTTT ATCGCAACGA GCGCATCCAC AAGGCCGTGA GCGTGAGCCG  
 GACCCGCAAA TAGCGTTGCT CGCGTAGGTG TTCCGGCACT CGCACTCGGC  
 10701 GCGGCGCGAG CTCAGCGACC GCGAGCTGAT GCACAGCCTG CAAAGGGCCC  
 CGCCGCGCTC GAGTCGCTGG CGCTCGACTA CGTGTCGGAC GTTTCGCGG  
 10751 TGGCTGGCAC GGGCAGCGGC GATAGAGAGG CCGAGTCTTA CTTTGACGCG  
 ACCGACCGTG CCCGTCGCCG CTATCTCTCC GGCTCAGGAT GAAACTGCGC  
 10801 GCGCTGACC TGCGCTGGGC CCCAAGCCGA CGCGCCCTGG AGGCAGCTGG  
 CCGCGACTGG ACGCGACCCG GGGTTCGGCT GCGCGGACC TCCGTCGACC  
 10851 GGCCGGACCT GGGCTGGCGG TGGCACCCGC GCGCGCTGGC AACGTCGGCG  
 CCGGCCTGGA CCCGACCGCC ACCGTGSGCG CGCGCGACCG TTGCAGCCGC  
 10901 GCGTGAGGA ATATGACGAG GACGATGAGT ACGAGCCAGA GGACGGCGAG  
 CGCACCTCCT TATACTGCTC CTGCTACTCA TGCTCGGTCT CCTGCCGCTC  
 10951 TACTAAGCGG TGATGTTTCT GATCAGATGA TGCAAGACGC AACGGACCCG  
 ATGATTGCC ACTACAAAGA CTAGTCTACT ACGTCTGCG TTGCTGGGC  
 11001 GCGGTGCGGG CGGCGCTGCA GAGCCAGCCG TCCGGCCTTA ACTCCACGGA  
 CGCCACGCCC GCGCGACGT CTCGTCGGC AGGCCGAAT TGAGGTGCCT  
 11051 CGACTGGCGC CAGGTCATGG ACCGCATCAT GTCGCTGACT GCGCGCAATC  
 GCTGACCGCG GTCCAGTACC TGGCGTAGTA CAGCGACTGA CGCGCGTTAG  
 11101 CTGACGCGTT CCGGCAGCAG CCGCAGGCCA ACCGGCTCTC CGCAATTCTG  
 GACTGCGCAA GCGCTCGTC GCGTCCGGT TGGCCGAGAG GCGTTAAGAC  
 11151 GAAGCGGTGG TCCCGGCGCG CGCAAACCCG ACGCACGAGA AGGTGCTGGC  
 CTTGCGCAC AGGGCCGCGC GCGTTGGGG TGCGTGCTCT TCCACGACCG  
 11201 GATCGTAAAC GCGCTGGCCG AAAACAGGGC CATCCGGCCC GACGAGGCCG  
 CTAGCATTTG CGCGACCGC TTTTGTCCCG GTAGGCCGGG CTGCTCCGGC  
 11251 GCCTGGTCTA CGACGCGCTG CTTACGCGCG TGGCTCGTTA CAACAGCGGC  
 CCGACCAGAT GTCGCGGAC GAAGTCGCGC ACCGAGCAAT GTTGTCGCGC

Figure 27L

11301 AACGTGCAGA CCTTGGG CCGGCTGGTG GGGGATGTGC GCGAGGCTT  
 TTGCACGTCT GGTGGACCT GGCCGACCAC CCCCTACACG CGCTCCGCA  
 11351 GGCGCAGCGT GAGCGCGCGC AGCAGCAGGG CAACCTGGGC TCCATGGTTG  
 CCGCGTCGCA CTCGCGCGCG TCGTCGTCCC GTTGGACCCG AGGTACCAAC  
 11401 CACTAAACGC CTTCTGAGT ACACAGCCCG CCAACGTGCC GCGGGGACAG  
 GTGATTTGCG GAAGGACTCA TGTGTCGGGC GGTGACACGG CGCCCTGTG  
 11451 GAGGACTACA CCAACTTTGT GAGCGCACTG CGGCTAATGG TGA CTGAGAC  
 CTCCTGATGT GGTGAAACA CTCGCGTGAC GCCGATTACC ACTGACTCTG  
 11501 ACCGCAAAGT GAGGTGTACC AGTCTGGGCC AGACTATTTT TTCCAGACCA  
 TGGCGTTTCA CTCACATGG TCAGACCCGG TCTGATAAAA AAGGTCTGGT  
 11551 GTAGACAAGG CTTGCAGACC GTAAACCTGA GCCAGGCTTT CAAAACTTG  
 CATCTGTTC GAGCGTCTGG CATTTGACT CGGTCCGAAA GTTTTTGAAC  
 11601 CAGGGGCTGT GGGGGGTGCG GGCTCCACA GCGGACCGCG CGACCGTGTG  
 GTCCCCGACA CCCCCACGC CCGAGGGTGT CCGCTGGCGC GCTGGCACAG  
 11651 TAGCTTGCTG ACGCCCAACT CGCGCCTGTT GCTGCTGCTA ATAGCGCCCT  
 ATCGAACGAC TGGGGGTGTA GCGCGGACAA CGACGACGAT TATCGCGGGA  
 11701 TCACGGACAG TGGCAGCGTG TCCCGGGACA CATACTAGG TCACTTGCTG  
 AGTGCTGTG ACCGTGCGAC AGGGCCCTGT GTATGGATCC AGTGAACGAC  
 11751 ACACTGTACC GCGAGGCCAT AGGTCAGGCG CATGTGGACG AGCATACTTT  
 TGTGACATGG CGCTCCGGTA TCCAGTCCGC GTACACCTGC TCGTATGAAA  
 11801 CCAGGAGATT ACAAGTGTCA GCCGCGCGCT GGGGCAGGAG GACACGGGCA  
 GGTCTCTTAA TGTTCACAGT CCGCGCGCGA CCCCCTCCTC CTGTGCCCGT  
 11851 GCCTGGAGGC AACCTAAAC TACCTGCTGA CCAACCGGCG GCAGAAGATC  
 CGGACCTCCG TTGGGATTG ATGGACGACT GGTGGCCGC CGTCTTCTAG  
 11901 CCCTCGTTGC ACAGTTTAA CAGCGAGGAG GAGCGCATTT TCGCTACGT  
 GGGAGCAACG TGTCAAATTT GTCGCTCCTC CTCGCGTAAA ACGCGATGCA  
 11951 GCAGCAGAGC GTGAGCCTTA ACCTGATGCG CGACGGGGTA ACGCCAGCG  
 CGTCGTCTCG CACTCGGAAT TGGACTACGC GCTGCCCCAT TCGGGGTGCG  
 12001 TGGCGCTGGA CATGACCGCG CGCAACATGG AACCGGGCAT GTATGCCTCA  
 ACCGCGACCT GTACTGGCGC GCGTTGTACC TTGGCCCGTA CATACGGAGT  
 12051 AACCGGCCGT TTATCAACCG CCTAATGGAC TACTTGATC GCGCGGCCGC  
 TTGGCCGGCA AATAGTTGGC GGATTACCTG ATGAACGTAG CGCGCCGGCG  
 12101 CGTGAACCCC GAGTATTTCA CCAATGCCAT CTTGAACCCG CACTGGCTAC  
 GCACTTGGGG CTCATAAAGT GGTACGGTA GAACTTGGGC GTGACCGATG  
 12151 CGCCCCCTGG TTTCTACACC GGGGGATTG AGGTGCCCGA GGSTAACGAT  
 GCGGGGGACC AAAGATGTGG CCCCCTAAGC TCCACGGGCT CCCATTGCTA  
 12201 GGATTCTCT GGGACGACAT AGACGACAGC GTGTTTTCCC CGCAACCGCA  
 CCTAAGGAGA CCCTGCTGTA TCTGCTGTG CACAAAAGGG GCGTTGGCGT

Figure 27 M

12251 GACCCCTGCTA GATTGCAAC AGCGCGAGCA GGCAGAGGCG GCGCTGCTAA  
 CTGGGACGAT CTAACGTTG TCGCGCTCGT CCGTCTCCGC CGCGACCTT

12301 AGGAAAGCTT CCGCAGGCCA AGCAGCTTGT CCGATCTAGG CGCTGCGGCC  
 TCCTTTCGAA GCGTCCGGT TCGTCGAACA GGCTAGATCC GCGACGCCGG

12351 CCGCGGTGAG ATGCTAGTAG CCCATTTCCA AGCTTGATAG GGTCTCTTAC  
 GGCGCCAGTC TACGATCATC GGGTAAAGGT TCGAATATC CCAGAGAATG

12401 CAGCACTCGC ACCACCCGCC CGCGCCTGCT GGGCGAGGAG GAGTACCTAA  
 GTCGTGAGCG TGGTGGGCGG GCGCGGACGA CCCGCTCCTC CTCATGGATT

12451 ACAACTCGCT GCTGCAGCCG CAGCGCGAAA AAAACCTGCC TCCGGCATT  
 TGTGAGCGA CGACGTCGGC GTCGCGCTTT TTTTGGACGG AGGCCGTAA

12501 CCCAACAAACG GGATAGAGAG CCTAGTGGAC AAGATGAGTA GATGGAAGAC  
 GGGTTGTTGC CCTATCTCTC GGATCACCTG TTCTACTCAT CTACCTTCTG

12551 GTACGCGCAG GAGCACAGGG ACGTGCCAGG CCCGCGCCCG CCCACCCGTC  
 CATGCGCGTC CTCGTGTCCC TGCACGGTCC GGGCGCGGGC GGGTGGGCAG

12601 GTCAAAGGCA CGACCGTCAG CGGGGTCTGG TGTGGGAGGA CGATGACTCG  
 CAGTTTCCGT GCTGGCAGTC GCCCAGACC ACACCTCCT GCTACTGAGC

12651 GCAGACGACA GCAGCGTCCT GGATTGCGA GGGAGTGGCA ACCCGTTTGC  
 CGTCTGCTGT CGTCGCAGGA CCTAAACCT CCCTCACCGT TGGGCAAACG

12701 GCACCTTCGC CCCAGGCTGG GGAGAATGTT TTAACAAAAA AAAAAGCATG  
 CGTGGAAGCG GGGTCCGACC CCTCTTACAA AATTTTTTTT TTTTTCGTAC

12751 ATGCAAAATA AAAAATCAC CAAGGCCATG GCACCGAGCG TTGGTTTCT  
 TACGTTTTAT TTTTGTAGTG GTTCCGCTAC CGTGGCTCGC AACCAAAGA

12801 TGTATTCCCC TTAGTATGCG GCGCGCGGCG ATGTATGAGG AAGGTCCTCC  
 ACATAAGGGG AATCATAACG CGCGCGCCGC TACATACTCC TTCCAGGAGG

12851 TCCCTCTTAC GAGAGTGTGG TGAGCGCGGC GCCAGTGGCG GCGGCGCTGG  
 AGGGAGGATG CTCTCACACC ACTCGCGCCG CGGTACCCGC CGCCGCGACC

12901 GTTCTCCCTT CGATGCTCCC CTGGACCCGC CGTTTGTGCC TCCGCGGTAC  
 CAAGAGGGAA GCTACGAGGG GACCTGGGCG GCAAACACGG AGGCGCCATG

12951 CTGCGGCCCTA CCGGGGGGAG AAACAGCATC CGTTACTCTG AGTTGGCACC  
 GACGCCGGAT GGCCCCCTC TTTGTCTAG GCAATGAGAC TCAACCGTGG

13001 CCTATTTCGAC ACCACCCGTG TGTACCTGGT GGACAACAAG TCAACGGATG  
 GGATAAGCTG TGGTGGGCAC ACATGGACCA CCTGTGTGTT AGTTGCCTAC

13051 TGGCATCCCT GAACTACCAG AACGACCACA GCAACTTTCT GACCACGGTC  
 ACCGTAGGGA CTTGATGGTC TTGCTGGTGT CGTTGAAAGA CTGGTGCCAG

13101 ATTCAAAACA ATGACTACAG CCCGGGGGAG GCAAGCACAC AGACCATCAA  
 TAAGTTTTGT TACTGATGTC GGGCCCCCTC CGTTCTGTGT TCTGGTAGTT

13151 TCTTGACGAC CGGTCGCACT GGGGCGGCGA CCTGAAAACC ATCCTGCATA  
 AGAACTGCTG GCCAGCGTGA CCCCAGCGCT GGACTTTTGG TAGGACGTAT

Figure 27N

13201 CCAACATGCC AATGTTGAAC GAGTTCATGT TTACCAATAA GTTTAAGCGG  
 GGTGTGACCG TTTCACTTG CTCAAGTACA AATGGTTATT CAAATTTC  
 13251 CGGGTGATGG TGTGCGCCTT GCCTACTAAG GACAATCAGG TGGAGCTGAA  
 GCCCACTACC ACAGCGCGAA CGGATGATTC CTGTTAGTCC ACCTCGACTT  
 13301 ATACGAGTGG GTGGAGTTCA CGCTGCCCCG GGGCAACTAC TCCGAGACCA  
 TATGCTCACC CACCTCAAGT GCGACGGGCT CCCGTTGATG AGGCTCTGGT  
 13351 TGACCATAGA CCTTATGAAC AACGCGATCG TGGAGCACTA CTTGAAAGTG  
 ACTGGTATCT GGAATACTTG TTGCGCTAGC ACCTCGTGAT GAACTTTTAC  
 13401 GGCAGACAGA ACGGGGTTCT GGAAAGCGAC ATCGGGGTAA AGTTTGACAC  
 CCGTCTGTCT TGCCCCAAGA CCTTTCGCTG TAGCCCCATT TCAAACGTGTG  
 13451 CCGCAACTTC AGACTGGGGT TTGACCCCGT CACTGGTCTT GTCATGCCTG  
 GGCGTTGAAG TCTGACCCCA AACTGGGGCA GTGACCAGAA CAGTACGGAC  
 13501 GGGTATATAC AAACGAAGCC TTCCATCCAG ACATCATTTT GCTGCCAGGA  
 CCCATATATG TTTGCTTCGG AAGGTAGGTC TGTAGTAAAA CGACGGTCTT  
 13551 TGCGGGGTGG ACTTCACCCA CAGCCGCCTG AGCAACTTGT TGGGCATCCG  
 ACGCCCCACC TGAAGTGGGT GTCGGCGGAC TCGTTGAACA ACCCGTAGGC  
 13601 CAAGCGGCAA CCCTTCCAGG AGGGCTTTAG GATCACCTAC GATGATCTGG  
 GTTCGCCGTT GGAAGGTCC TCCCGAAATC CTAGTGGATG CTACTAGACC  
 13651 AGGGTGGTAA CATTCCCGCA CTGTTGGATG TGGACGCCTA CCAGGCGAGC  
 TCCCACCATT GTAAGGCGGT GACAACCTAC ACCTGCGGAT GGTCCGCTCG  
 13701 TTGAAAGATG ACACCGAACA GGGCGGGGGT GCGCGAGGCG GCAGCAACAG  
 AACTTTCTAC TGTGGCTTGT CCCGCCCCCA CCGCGTCCGC CGTCGTTGTG  
 13751 CAGTGGCAGC GCGCGGAAG AGAACTCCAA CGCGGCAGCC GCGGCAATGC  
 GTCACCGTCG CCGCGCCTTC TCTTGAGGTT GCGCGGTCGG CGCCGTACG  
 13801 AGCCGGTGGA GGACATGAAC GATCATGCCA TTCGCGGCGA CACCTTTGCC  
 TCGGCCACCT CCTGTACTTG CTAGTACGGT AAGCGCCGCT GTGGAAACGG  
 13851 ACACGGGCTG AGEAGAAGCG CGCTGAGGCC GAAGCAGCGG CCGAAGCTGC  
 TGTGCCCCAC TCCTCTTCGC GCGACTCCGG CTTGTCGCC GGCTTCGACG  
 13901 CGCCCCGCT GCGCAACCCG AGGTCGAGAA GCCTCAGAAG AAACCGGTGA  
 GCGGGGGCGA CGCGTTGGGC TCCAGCTCTT CGGAGTCTTC TTTGGCCACT  
 13951 TCAAACCCCT GACAGAGGAC AGCAAGAAAC GCAGTTACAA CCTAATAAGC  
 AGTTTGGGGA CTGTCTCCTG TCGTTCTTTG CGTCAATGTT GGATTATTG  
 14001 AATGACAGCA CCTTCACCCA GTACCGCAGC TGGTACCTTG CATACAACTA  
 TTACTGTCTG GGAAGTGGGT CATGGCGTCG ACCATGGAAC GTATGTTGAT  
 14051 CGGCGACCTT CAGACCGGAA TCCGCTCATG GACCTGCTT TGCCTCTCTG  
 GCCGCTGGGA GTCTGGCCTT AGGCGAGTAC CTGGGACGAA ACCTGAGGAC  
 14101 ACGTAACCTG CGGCTCGGAG CAGGTCTACT GGTGCTTCCC AGACATGATG  
 TGCATTGGAC GCGGAGCTC GTCCAGATGA CCAGCAACGG TCTGTACTAC

Figure 270

14151 CAAGACCCCG TTTTCCG CTCCACGCGC CAGATCAGCA ACTTTCCT  
 GTTCTGGGGC ACTGGAAGGC GAGGTGCGCG GTCTAGTCGT TGAAAGGCTA  
 14201 GGTGGGCGCC GAGCTGTTGC CCGTGCACTC CAAGAGCTTC TACAACGACC  
 CCACCCGCGG CTCGACAACG GGCACGTGAG GTTCTCGAAG ATGTTGCTGG  
 14251 AGGCCGTCTA CTCCCAACTC ATCCGCCAGT TTACCTCTCT GACCCACGTG  
 TCCGGCAGAT GAGGCTTGAG TAGGCGGTCA AATGGAGAGA CTGGGTGCAC  
 14301 TTCAATCGCT TTCCCGAGAA CCAGATTTTG GCGCGCCCGC CAGCCCCCAG  
 AAGTTAGCGA AAGGGCTCTT GGTCTAAAC CCGCGGGGCG GTCGGGGGTG  
 14351 CATCACCACC GTCAGTGAAG ACCTTCCTGC TCTCACAGAT CACGGGACGC  
 GTAGTGGTGG CAGTCACTTT TGCAAGGACG AGAGTGTCTA GTGCCCTGCG  
 14401 TACCGCTCGG CAACAGCATC GGAGGAGTCC AGCGAGTGAC CATTACTGAC  
 ATGGCGACGC GTTGTCTGAG CCTCCTCAGG TCGCTCACTG GTAATGACTG  
 14451 GCCAGACGCC GCACCTGCCC CTACGTTTAC AAGGCCCTGG GCATAGTCTC  
 CGGTCTGCGG CGTGGACGGG GATGCAAAATG TTCCGGGACC CGTATCAGAG  
 14501 GCCGCGCGTC CTATCGAGCC GCACTTTTTG AGCAAGCATG TCCATCCTTA  
 CGGCGCGCAG GATAGCTCGG CGTGAATAAC TCCTTCGTAC AGGTAGGAAT  
 14551 TATCGCCAG CAATAACACA GGCTGGGGCC TCGCTTCCC AAGCAAGATG  
 ATAGCGGGTC GTTATTGTGT CCGACCCCGG ACGCGAAGGG TTCGTTCTAC  
 14601 TTTGGCGGGG CCAAGAAGCG CTCCGACCAA CACCCAGTGC GCGTGCAGCG  
 AAACCGCCCC GGTCTTTCGC GAGGCTGGTT GTGGGTACAG CGCACGCGCC  
 14651 GCACTACCGC GCGCCTTGGG GCGCGCACAA ACGCGGCCGC ACTGGGCGCA  
 CGTGATGGCG CGCGGGACCC CGCGCGTGT TCGCGCGGCG TGACCCCGCT  
 14701 CCACCGTCGA TGACGCCATC GACCGCGTGG TGGAGGAGGC GCGCAACTAC  
 GGTGGCAGCT ACTGCGGTAG CTGCGCCACC ACCTCCTCCG CGCGTTGATG  
 14751 ACGCCACGCG CGCCACCAGT GTCCACAGTG GACGCGGCCA TTCAGACCGT  
 TCGGGGTGCG GCGGTGGTCA CAGGTGTAC CTGCGCCGGT AAGTCTGGCA  
 14801 GGTGCGCGGA GCCCGGCGCT ATGCTAAAAT GAAGAGACGG CGGAGGCGCG  
 CCACGCGCCT CGGGCCGCGA TACGATTITA CTCTCTGCC GCCTCCGCGC  
 14851 TAGCACGTCG CCACCGCCGC CGACCCGSCA CTGCGGCCCA ACGCGCGGCG  
 ATCGTGACAG GGTGGCGGCG GCTGGGCGGT GACGCGGGGT TGCGCGCCGC  
 14901 GCGGCCCTGC TTAACCGCGC ACGTCGCACC GGCCGACGGG CGGCCATGCG  
 CGCCGGGACG AATTGGCGCG TGCAGCGTGG CCGGCTGCCC GCCGGTACGC  
 14951 GCGCGCTCGA AGGCTGGCCG CGGGTATTGT CACTGTGCCC CCCAGGTCCA  
 CCGGCGAGCT TCCGACCGGC GCCCATAACA GTGACACGGG GGGTCCAGGT  
 15001 GCGGACGAGC GGCGGCGGCA GCAGCCGCGG CCATTAGTGC TATGACTCAG  
 CCGCTGCTCG CCGGCGGCGT CGTCGGCGCC GGTAAATCAG ATACTGAGTC  
 15051 GGTGCGAGGG GCAACGTGTA TTGGGTGCGC GACTCGGTTA GCGGCCTGCG  
 CCAGCGTCCC CGTTGCACAT AAGCCACGCG CTGAGCCAAT CGCCGACGCG

Figure 27P

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15101 CGTGCCCGTG CCCCCCGCC CCCC GCGCAA CTAGATTGCA TGA AAAAT
GCACGGGCAC GCGGGGCGG GGGGCGCCTT GATCTAACGT TCTTTTGA

15151 ACTTAGACTC GTACTGTTGT ATGTATCCAG CGGCGGCGGC GCGCAACGAA
TGAATCTGAG CATGACAACA TACATAGGTC GCCCGCGCCG CGCGTTGCTT

15201 GCTATGTCCA AGCGCAAAAT CAAAGAAGAG ATGCTCCAGG TCATCGCGCC
CGATACAGGT TCGCGTTTGA GTTCTTCTC TACGAGGTCC AGTAGCGCGG

15251 GGAGATCTAT GGCCCCCGA AGAAGGAAGA GCAGGATTAC AAGCCCCGAA
CCTCTAGATA CCGGGGGGCT TCTTCTTCT CGTCTAATG TTCGGGGCTT

15301 AGCTAAAGCG GGTCAAAAG AAAAAGAAAG ATGATGATGA TGA ACTTGAC
TCGATTTGCG CCAGTTTTC TTTTCTTTC TACTACTACT ACTTGA ACTG

15351 GACGAGGTGG AACTGCTGCA CGCTACCGCG CCCAGGCGAC GGGTACAGTG
CTGCTCCACC TTGACGACGT GCGATGGCGC GGGTCCGCTG CCCATGTCAC

15401 GAAAGGTCTGA CGCGTAAAC GTGTTTTCG ACCCGGCACC ACCGTAGTCT
CTTTCAGCT CGGCATTTC CACAAAACGC TGGGCGTGG TGGCATCAGA

15451 TTACGCCCCG TGAGCGCTCC ACCCGCACCT ACAAGCGCGT GTATGATGAG
AATGCGGGCC ACTCGCGAGG TGGGCGTGA TGTTCGCGCA CATACTACTC

15501 GTGTACGGCG ACGAGGACCT GCTTGAGCAG GCCAACGAGC GCCTCGGGGA
CACATGCCGC TGCTCCTGGA CGAACTCGTC CGGTTCGCTC CGGAGCCCTT

15551 GTTTCCTTAC GGAAAGCGGC ATAAGGACAT GCTGGCGTTG CCGCTGGACG
CAAACGGATG CCTTTCGCGG TATTCTGTA CGACCGCAAC GGCACCTGC

15601 AGGGCAACCC AACACCTAGC CTAAAGCCCG TAACACTGCA GCAGGTGCTG
TCCCGTTGGG TTGTGGATCG GATTTCGGGC ATTGTGACGT CGTCCACGAC

15651 CCCGCGCTTG CACCGTCCGA AGAAAAGCGC GGCTAAAGC GCGAGTCTGG
GGGCGCAAC GTGGCAGGCT TCTTTTCGCG CCGGATTTCG CGCTCAGACC

15701 TGACTTGGCA CCCACCGTGC AGCTGATGGT ACCCAAGCGC CAGCGACTGG
ACTGAACCGT GGGTGGCAGC TCGACTACCA TGGGTTGCGG GTCGCTGACC

15751 AAGATGTCTT GGAAAAATG ACCGTGGAAC CTGGGCTGGA GCGCGAGGTC
TTCTACAGAA CCTTTTTC TGGCACCTTG GACCCGACCT CGGGCTCCAG

15801 CGCGTGCGGC CAATCAAGCA GGTGGCGCGG GGACTGGGCG TCAGACCGT
GCGCACGCGG GTTAGTTCGT CCACCGCGG CCTGACCGC AGTCTGGCA

15851 GGACGTTTCTG ATACCCACTA CCACTAGCAC CAGTATTGCC ACCGCCACAG
CCTGCAAGTC TATGGGTGAT GGTATCTGTC GTCATAACGG TGGCGGTGTC

15901 AGGGCATGGA GACACAAAG TCCCGCGTTG CCTCAGCGGT GCGGATGCC
TCCCGTACCT CTGTGTTTGC AGGGGCCAAC GGAGTCGCA CCGCTACGG

15951 GCGGTGCAGG CGGTGCTGTC GGCCGCGTCC AAGACCTCTA CGGAGGTGCA
CGCCACGTCC GCCAGCGAGC CCGCGCAGG TTCTGGAGAT GCCTCCACGT

16001 AACGGACCCG TGGATGTTTC GCGTTTCAGC CCCCCGCGC CCGCGCGGTT
TTGCTTGGG ACCTACAAAG CGCAAAGTC GGGGCGCGG GCGCGGCA

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Figure 27A

16051 CGAGGAAGTA CCGGCGGCC AGCGCGCTAC TGCCCGAATA TGCCCTAATT  
GCTCCTTCAT GCCGCGGCGG TCGCGCGATG ACGGGCTTAT ACGGGATGTA

16101 CCTTCCATTG CGCCTACCCC CGGCTATCGT GGCTACACCT ACCGCCCCAG  
GGAAGGTAAC GCGGATGGGG GCCGATAGCA CCGATGTGGA TGGCGGGGTC

16151 AAGACGAGCA ACTACCCGAC GCCGAACCAC CACTGGAACC CGCCCGCGCC  
TTCTGCTCGT TGA TGGGCTG CGGCTTGGTG GTGACCTTGG GCGGCGGCGG

16201 GTCGCCGTG CCAGCCCGTG CTGGCCCCGA TTTCCGTGCG CAGGGTGGCT  
CAGCGGCAGC GGTGCGGCAC GACCGGGGCT AAAGGCACGC GTCCACCGA

16251 CGCGAAGGAG GCAGGACCCT GGTGCTGCCA ACAGCGCGCT ACCACCCAG  
GCGCTTCCTC CGTCCTGGGA CCACGACGGT TGTCGCGCGA TGGTGGGGTC

16301 CATCGTTTAA AAGCCGGTCT TTGTGGTTCT TGCAGATATG GCCCTCACCT  
GTAGCAAATT TTCGGCCAGA AACACCAAGA ACGTCTATAC CGGGAGTGGA

16351 GCCGCCTCCG TTTCCCGGTG CCGGGATTCC GAGGAAGAAT GCACCGTAGG  
CGGCGGAGGC AAAGGGCCAC GGCCTTAAGG CTCTTCTTA CGTGGCATCC

16401 AGGGGCATGG CCGGCCACGG CCTGACGGGC GGCATGCGTC GTGCGCACCA  
TCCCCGTACC GGCCGGTGCC GGACTGCCCG CCGTACGCAG CACCGTGGT

16451 CCGGCGGCGG CGCGCGTGC ACCGTGCGAT GCGCGCGGGT ATCCTGCCCC  
GGCCGCCGCC GC CGCGCAGCG TGGCAGCGTA CCGCGCCGCA TAGGACGGGG

16501 TCCTTATTC ACTGATCGCC GCGGCGATTG GCGCGTGCC CGGAATTGCA  
AGGAATAAGG TGACTAGCGG CGCCGCTAAC CCGCGCACGG GCCTTAACGT

16551 TCCGTGGCCT TGCAGGCGCA GAGACACTGA TTAAAAACAA GTTGCAATGTG  
AGGCACCGGA ACGTCCGCGT CTCTGTGACT AATTTTGT CAACGTACAC

16601 GAAAAATCAA AATAAAAGT CTGGACTCTC ACGCTCGCTT GGTCTGTAA  
CTTTTATGTT TTATTTTCA GACCTGAGAG TCGGAGCGAA CCAGGACATT

16651 CTATTTTGTA GAATGGAAGA CATCAACTTT GCGTCTCTGG CCCC GCGACA  
GATAAAACAT CTACCTTCT GTAGTTGAAA CGCAGAGACC GGGGCGCTGT

16701 CGGCTCGCGC CCGTTCATGG GAAACTGGCA AGATATCGGC ACCAGCAATA  
GCCGAGCGCG GGCAAGTACC CTTTGACCGT TCTATAGCCG TGGTCGTTAT

16751 TGAGCGGTGG CGCCTTCAGC TGGGGCTCGC TGTGGAGCGG CATTAAAAAT  
ACTCGCCACC GCGGAAGTCG ACCCGGAGCG ACACCTCGCC GTAATTTT

16801 TTCGGTTCCA CCGTTAAGAA CTATGGCAGC AAGGCCTGGA ACAGCAGCAC  
AAGCCAAGGT GGCAATTCTT GATACCGTCG TTCCGGACCT TGTCGTCGTG

16851 AGGCCAGATG CTGAGGGATA AGTTGAAAGA GCAAAATTTC CAACAAAAGG  
TCCGGTCTAC GACTCCCTAT TCACTTTCT CGTTTTAAAG GTTGTTCCTC

16901 TGGTAGATGG CCTGGCCTCT GGCATTAGCG GGGTGGTGA CCTGGCCAAC  
ACCATCTACC GGACCGGAGA CCGTAATCGC CCCACCACCT GGACCGGTTG

16951 CAGGCAGTGC AAAATAAGAT TAACAGTAAG CTTGATCCCC GCCCTCCCGT  
GTCCGTACG TTTTATTCTA ATTGTCATTC GAACTAGGGG CGGGAGGGCA

Figure 27R



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17001 AGAGGAGCCT CCGGCCG TGGAGACAGT GTCTCCAGAG GGGCGTGG
      TCTCCTCGGA GGTGGCCGGC ACCTCTGTCA CAGAGGTCTC CCCGCACCGC

17051 AAAAGCGTCC GCGCCCCGAC AGGGAAGAAA CTCTGGTGAC GCAAATAGAC
      TTTTCGCAGG CGCGGGGCTG TCCCTTCTTT GAGACCACTG CGTTTATCTG

17101 GAGCCTCCCT CGTACGAGGA GGCACATAAG CAAGGCCTGC CCACCACCCG
      CTCGGAGGGA GCATGCTCCT CCGTGTCTTC GTTCCGGACG GGTGGTGGGC

17151 TCCCATCGCG CCCATGGCTA CCGGAGTGCT GGGCCAGCAC ACACCCGTAA
      AGGGTAGCGC GGTACCAGAT GGCTCACGA CCCGGTCGTG TGTGGGCATT

17201 CGCTGGACCT GCCTCCCCC GCCGACACCC AGCAGAAACC TGTGCTGCCA
      GCGACCTGGA CGGAGGGGGG CGGCTGTGGG TCGTCTTTGG ACACGACGGT

17251 GGGCCGACCG CCGTTGTTGT AACCCTGCTT AGCCGCGCGT CCCTGCGCCG
      CCGGGCTGGC GGAACAACA TTGGGCAGGA TCGGCGCGCA GGGACGCGCG

17301 CGCCGCCAGC GGTCCCGCAT CGTTGCGGCC CGTAGCCAGT GGCAACTGGC
      GCGGCGGTG CAGGCGCTA GCAACGCCGG GCATCGGTCA CCGTTGACCG

17351 AAAGCACACT GAACAGCATC GTGGGTCTGG GGGTGCAATC CCTGAAGCGC
      TTTCTGTGTA CTGTCTGTAG CACCCAGACC CCCACGTTAG GGACTTCGCG

17401 CGACGATGCT TCTGATAGCT AACGTGTCGT ATGTGTGTCA TGTATGCGTC
      GCTGCTACGA AGACTATCGA TTGCACAGCA TACACACAGT ACATACGCAG

17451 CATGTCGCCG CCAGAGGAGC TGCTGAGCCG CCGCGCGCCC GCTTTCCAAG
      GTACAGCGGC GGTCTCCTCG ACGACTCGGC GCGCGCGGG CAAAGGTTT

17501 ATGGCTACCC CTTCGATGAT GCCGAGTGG TCTTACATGC ACATCTCGGG
      TACCGATGGG GAAGCTACTA CGGCGTCACC AGAATGTACG TGTAGAGCCC

17551 CCAGGACGCC TCGGAGTACC TGAGCCCCGG GCTGGTGACG TTTGCCCGCG
      GGTCTGCGG AGCCTCATGG ACTCGGGGCC CGACCACGTC AAACGGGCGC

17601 CCACCGAGAC GTACTTCAGC CTGAATAACA AGTTTAGAAA CCCACGGTG
      GGTGGCTCTG CATGAAGTCG GACTTATTGT TCAAATCTTT GGGGTGCCAC

17651 GCGCCTACGC ACCACGTGAC CACAGACCGG TCCAGCGTT TACGCTGCG
      CGCGGATGCG TGCTGCACTG GTGTCTGACC AGGGTCGCAA ACTGCGACGC

17701 GTTCATCCCT GTGGACCGTG AGGATACTGC GTACTCGTAC AAGGCGCGGT
      CAAGTAGGGA CACCTGGCAC TCTATGACG CATGAGCATG TTCCGCGCCA

17751 TCACCTTAGC TGTTGGTGAT AACCGTGTGC TGGACATGGC TTCCACGTAC
      AGTGGGATCG ACACCCACTA TTGGCACACG ACCTGTACCG AAGGTGCATG

17801 TTTGACATCC GCGGCGTGCT GGACAGGGGC CCTACTTTTA AGCCCTACTC
      AAATGTAGG CGCCGCACGA CCGTCCCCG GGATGAAAAT TCGGGATGAG

17851 TGGCACTGCC TACAACGCCC TGGCTCCCAA GGGTGCCCCA AATCCTTGCG
      ACCGTGACGG ATGTTGCGGG ACCGAGGGT CCCACGGGGT TTAGGAACGC

17901 AATGGGATGA AGCTGCTACT GCTCTTGAAA TAAACCTAGA AGAAGAGGAC
      TTACCTACT TCGACGATGA CGAGAACTTT ATTTGGATCT TCTTCTCCTG

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Figure 275

17951 GATGACAACG ACGAAGT AGACGAGCAA GCTGAGCAGC AAAAAA  
 CTACTGTTGC TTCTGCTTCA TCTGCTCGTT CGACTCGTCG TTTTGTGAGT  
 18001 CGTATTTGGG CAGGCGCCTT ATTCTGGTAT AAATATTACA AAGGAGGGTA  
 GCATAAACCC GTCCGCGGAA TAAGACCATA TTTATAATGT TTCTCCCAT  
 18051 TTCAAATAGG TGTGGAAGGT CAAACACCTA AATATGCCGA TAAAACATTT  
 AAGTTTATCC ACAGCTTCCA GTTGTGGAT TTATACGGCT ATTTTGTAAA  
 18101 CAACCTGAAC CTCAAATAGG AGAATCTCAG TGGTACGAAA CAGAAATTAA  
 GTTGGACTTG GAGTTTATCC TCTTAGAGTC ACCATGCTTT GTCTTTAATT  
 18151 TCATGCAGCT GGGAGAGTCC TAAAAAGAC TACCCCAATG AAACCATGTT  
 AGTACGTGCA CCTCTCAGG ATTTTTCCTG ATGGGGTTAC TTTGGTACAA  
 18201 ACGGTTTATA TGCAAAACCC ACAAATGAAA ATGGAGGGCA AGGCATTCTT  
 TGCCAAGTAT ACGTTTGGG TGTTTACTTT TACCTCCCGT TCCGTAAGAA  
 18251 GTAAAGCAAC AAAATGGAAA GCTAGAAAGT CAAGTGGAAA TGCAATTTT  
 CATTTCGTTG TTTTACCTTT CGATCTTCA GTTACCTTT ACGTTAAAA  
 18301 CTCAACTACT GAGGCAGCCG CAGGCAATGG TGATAACTTG ACTCCTAAAG  
 GAGTTGATGA CTCCGTCGGC GTCCGTTACC ACTATTGAAC TGAGGATTC  
 18351 TGGTATTGTA CAGTGAAGAT GTAGATATAG AAACCCAGA CACTCATATT  
 ACCATAACAT GTCACCTCTA CATCTATATC TTTGGGGTCT GTGAGTATAA  
 18401 TCTTACATGC CCACTATTAA GGAAGGTAAC TCACGAGAAC TAATGGGCCA  
 AGAATGTACG GGTGATAATT CCTTCCATTG AGTGCTCTTG ATTACCCGGT  
 18451 ACAATCTATG CCCAACAGGC CTAATTACAT TGCTTTTAGG GACAATTTTA  
 TGTTAGATAC GGGTTGTCCG GATTAATGTA ACGAAAATCC CTGTTAAAT  
 18501 TTGGTCTAAT GTATTACAAC AGCACGGGTA ATATGGGTGT TCTGGCGGGC  
 AACCAGATTA CATAATGTTG TCGTGCCCAT TATACCCACA AGACCGCCCG  
 18551 CAAGCATCGC AGTTGAATGC TGTGTAGAT TTGCAAGACA GAAACACAGA  
 GTTCGTAGCG TCAACTTACG ACAACATCTA AACGTTCTGT CTTTGTGTCT  
 18601 GCTTTCATAC CAGCTTTTGC TTGATTCCAT TGGTGATAGA ACCAGGTACT  
 CGAAAGTATG GTCGAAAACG AACTAAGSTA ACCACTATCT TGGTCCATGA  
 18651 TTTCTATGTG GAATCAGGCT GTTGACAGCT ATGATCCAGA TGTTAGAATT  
 AAAGATACAC CTTAGTCCGA CAACTGTCGA TACTAGGTCT ACAATCTTAA  
 18701 ATTGAAAAATC ATGGAACCTGA AGATGAACTT CCAAATTACT GCTTTCCACT  
 TAACTTTATG TACCTTGACT TCTACTTGAA GGTTAATGA CGAAAGGTGA  
 18751 GGGAGGTGTG ATTAATACAG AGACTCTTAC CAAGSTAAAA CCTAAAACAG  
 CCTCCACAC TAATTATGTC TCTGAGAATG GTTCCATTTT GGATTTTGTG  
 18801 GTCAGGAAAA TGGATGGGAA AAAGATGCTA CAGAATTTTC AGATAAAAAAT  
 CAGTCCTTTT ACCTACCTTT TTTCTACGAT GTCCTAAAAG TCTATTTTAA  
 18851 GAAATAAGAG TTGGAATAA TTTTGCCATG GAAATCAATC TAAATGCCAA  
 CTTTATCTC AACCTTTATT AAAACGGTAC CTTTAGTTAG ATTTACGGTT

Figure 27T

18901 CCTGTGGAGA AATTCCTGT ACTCCAACAT AGCGCTGTAT TTGCCCCA  
 GGACACCTCT TTAAAGGACA TGAGGTGTGA TCGCGACATA AACGGGCTGT  
 18951 AGCTAAAGTA CAGTCCTTCC AACGTAAAAA TTTCTGATAA CCCAAACACC  
 TCGATTTCAT GTCAGGAAGG TTGCATTTTT AAAGACTATT GGGTTTGTGG  
 19001 TACGACTACA TGAACAAGCG AGTGGTGGCT CCCGGGCTAG TGGACTGCTA  
 ATGCTGATGT ACTTGTTCGC TCACCACCGA GGGCCCGATC ACCTGACGAT  
 19051 CATTAACTTT GGAGCACGCT GGTCCCTTGA CTATATGGAC AACGTCAACC  
 GTAATTGGAA CCTCGTGC GAAGGGAAC GATATACCTG TTGCAGTTGG  
 19101 CATTAAACCA CCACCACAAT GCTGGCCTGC GCTACCGCTC AATGTTGCTG  
 GTAAATTGGT GGTGGCGTTA CGACCGGACG CGATGGCGAG TTACAACGAC  
 19151 GGCATATGGTC GCTATGTGCC CTTCACATC CAGGTGCCTC AGAAGTTCTT  
 CCGTTACCAG CGATACACGG GAAGGTGTAG GTCCACGGAG TCTTCAAGAA  
 19201 TGCCATTAAA AACCTCCTTC TCCTGCCGGG CTCATACACC TACGAGTGGG  
 ACCGTAATTT TTGGAGGAAG AGGACGGCCC GAGTATGTGG ATGCTCACCT  
 19251 ACTTCAGGAA GGATGTTAAC ATGGTTCGTC AGAGCTCCCT AGGAAATGAC  
 TGAAGTCCTT CCTACAATTG TACCAAGACG TCTCGAGGGA TCCTTTACTG  
 19301 CTAAGGGTTG ACGGAGCCAG CATTAAAGTTT GATAGCATTG GCCTTTACGC  
 GATTCCCAAC TGCCTCGGTC GTAATTCAAA CTATCGTAAA CGGAAATGCG  
 19351 CACCTTCTTC CCCATGGCCC ACAACACCGC CTCCACGCTT GAGGCCATGC  
 GTGGAAGAAG GGGTACCGGG TGTGTGGCG GAGGTGCGAA CTCCGGTACG  
 19401 TTAGAAACGA CACCAACGAC CAGTCCTTTA ACGACTATCT CTCCGCCGCC  
 AATCTTTGCT GTGTTGCTG GTCAGGAAAT TGCTGATAGA GAGGCGGCGG  
 19451 AACATGCTCT ACCCTATACC CGCCAACGCT ACCAACGTGC CCATATCCAT  
 TTGTACGAGA TGGGATATGG GCGGTTGCGA TGGTTGCACG GGTATAGGTA  
 19501 CCCCTCCCGC AACTGGGCGG CTTTCCGCGG CTGGGCTTTC ACGCGCCTTA  
 GGGGAGGGCG TTGACCCGCC GAAAGGCGCC GACCCGGAAG TGCGCGGAAT  
 19551 AGACTAAGGA AACCCTATCA CTGGGCTCGG GCTACGACCC TTATTACACC  
 TCTGATTCTT TTGGGGTAGT GACCCGAGCC CGATGCTGGG AATAATGTGG  
 19601 TACTCTGGCT CTATACCTTA CCTAGATGGA ACCTTTTACC TCAACACAC  
 ATGAGACCGA GATATGGGAT GGATCTACCT TGGAAAATGG AGTTGGTGTG  
 19651 CTTTAAGAAG GTGGCCATTA CTTTGAATC TTCTGTCAGC TGGCCTGGCA  
 GAAATTCTTC CACCGGTAAT GGAACTGAG AAGACAGTCG ACCGGACCGT  
 19701 ATGACCGCCT GCTTACCCCT AACGAGTTTG AAATTAAGCG CTCAGTTGAC  
 TACTGGCGGA CGAATGGGGG TTGCTCAAAC TTTAATTGCG GAGTCAACTG  
 19751 GGGGAGGGTT ACAACGTGTC CCAGTGTAACT ATGACCAAAG ACTGGTTCCT  
 CCCCTCCCAA TGTTGCAACG GGTCAATG TACTGGTTTC TGACCAAGGA  
 19801 GGTACAAATG CTAGCTAACT ATAACATTGG CTACCAGGGC TTCTATATCC  
 CCATGTTTAC GATCGATTGA TATTGTAACC GATGGTCCCG AAGATATAGG

Figure 274

19851 CAGAGAGCTA C GACCGC ATGTACTCCT TCTTTAGAAA CTTCCAC  
GTCTCTCGAT GTTCCTGGCG TACATGAGGA AGAAATCTTT GAAGGTGCGG

19901 ATGAGCCGTC AGGTGGTGGA TGATACTAAA TACAAGGACT ACCAACAGGT  
TACTCGGCAG TCCACCACCT ACTATGATTT ATGTTCTCTGA TGGTTGTCCA

19951 GGGCATCCTA CACCAACACA ACAACTCTGG ATTTGTTGGC TACCTTGCCC  
CCCGTAGGAT GTGGTTGTGT TGTGAGACC TAAACAACCG ATGGAACGGG

20001 CCACCATGCG CGAAGGACAG GCCTACCCTG CTAACCTCCC CTATCCGCTT  
GGTGGTACGC GCTTCCTGTC CGGATGGGAC GATTGAAGGG GATAGCGCAA

20051 ATAGGCAAGA CCGCAGTTGA CAGCATTACC CAGAAAAAGT TTC'TTTGCGA  
TATCCGTTCT GCGTCAACT GTCGTAATGG GTCTTTTTC AAGAAACGCT

20101 TCGCACCTTT TGGCGCATCC CATTCTCCAG TAACTTTATG TCCATGGGCG  
AGCGTGGGAA ACCCGTAGG GTAAGAGGTC ATTGAAATAC AGGTACCCCG

20151 CACTCACAGA CCTGGGCCAA AACCTTCTCT ACGCCAATC CGCCACGCG  
GTGAGTGTCT GGACCCGGT TTGGAAGAGA TCGGTTGAG CGGGGTGCGC

20201 CTAGACATGA CTTTTGAGGT GGATCCCATG GACGAGCCCA CCTTCTTTA  
GATCTGTACT GAAAAC'TCCA CCTAGGGTAC CTGCTCGGGT GGGAGAAAT

20251 TGT'TTTGTTT GAAGTCTTTG ACGTGGTCCG TGTGCACCAG CCGCACGCG  
ACAAAACAAA CTTCAGAAAC TGCACCAGGC ACACGTGGTC GCGGTGCGC

20301 GCGTCATCGA AACCGTGTAC CTGCGCACGC CTTCTCGGC CGGCAACGCC  
CGCAGTAGCT TTGGCACATG GACGCGTGCG GGAAGAGCCG GCCGTGCGG

20351 ACAACATAAA GAAGCAAGCA ACATCAACAA CAGCTGCCGC CATGGGCTCC  
TGTGTATTT CTTCTTCGT TGTAGTTGTT GTCGACGGCG GTACCCGAGG

20401 AGTGAGCAGG AACTGAAAGC CATTGTCAAA GATCTTGGTT GTGGGCCATA  
TCACTCGTCC TTGACTTTCG GTAACAGTTT CTAGAACCAA CACCCGGTAT

20451 TTTT'TGGGC ACCTATGACA AGCGCTTTC AGGCTTTGTT TCTCCACACA  
AAAAAACCCG TGGATACTGT TCGCGAAAGG TCCGAAACAA AGAGGTGTGT

20501 AGCTCGCCTG CGCCATAGTC AATACGGCCG GTCGCGAGAC TGGGGGCGTA  
TCGAGCGGAC GCGGTATCAG TTATGCCGGC CAGCGCTCTG ACCCCGCGAT

20551 CACTGGATGG CTTTGCCTG GAACCCGCAC TCAAAAACAT GCTACCTCTT  
GTGACCTACC GGAACCGGAC CTTGGGCGTG AGTTTTTGTA CGATGGAGAA

20601 TGAGCCCTTT GGCTTTTCTG ACCAGCGACT CAAGCAGGTT TACCAGTTG  
ACTCGGGAAA CCGAAAAGAC TGGTCGCTGA GTTCGTCAA ATGGTCAAAC

20651 AGTACGAGTC ACTCTGCGC CGTAGCGCCA TTGCTTCTTC CCCCAGCCG  
TCATGCTCAG TGGGACGCG GCATCGCGGT AACGAAGAAG GGGGCTGGCG

20701 TGTATAACGC TGGAAAAGTC CACCCAAAGC GTACAGGGGC CCAACTCGGC  
ACATATTGCG ACCTTTCAG GTGGGTTTCG CATGTCCCCG GGTGAGCCG

20751 CGCTGTGGA CTATTCTGCT GCATGTTTCT CCACGCCTTT GCCAACTGGC  
CGGACACCT GATAAGACGA CGTACAAAGA GGTGCGGAAA CGTTGACCG

Figure 27V.

20801 CCCAACTCC C GATCAC AACCCACCA TGAACCTTAT TACCGG A  
 GGGTTTGAGG GTACCTAGTG TTGGGGTGGT ACTTGGAATA ATGGCCCCAT  
 20851 CCCAACTCCA TGCTCAACAG TCCCAGSTA CAGCCCACCC TGCGTCGCAA  
 GGGTTGAGGT ACGAGTTGTC AGGGGTCCAT GTCGGGTGGG ACGCAGCGTT  
 20901 CCAGGAACAG CTCTACAGCT TCCTGGAGCG CCACTCGCCC TACTTCCGCA  
 GGTCTTTGTC GAGATGTCGA AGGACCTCGC GGTGAGCGGG ATGAAGGCGT  
 20951 GCCACAGTGC GCAGATTAGG AGCGCCACTT CTTTTTGTCA CTTGAAAAAC  
 CGGTGTCACG CGTCTAATCC TCGCGGTGAA GAAAAACAGT GAACTTTTTG  
 21001 ATGTAAAAAT AATGTACTAG AGACACTTTC AATAAAGGCA AATGCTTTTA  
 TACATTTTTA TTACATGATC TCTGTGAAAG TTATTTCCGT TIACGAAAAAT  
 21051 TTTGTACACT CTCGGGTGAT TATTTACCCC CACCCCTTGCC GTCTGCGCCG  
 AAACATGTGA GAGCCCACTA ATAAATGGGG GTGGGAACGG CAGACGCGGC  
 21101 TTTAAAAATC AAAGGGGTTC TGCCGCGCAT CGCTATGCGC CACTGGCAGG  
 AAATTTTTAG TTTCCCAAG ACGGCGCGTA GCGATACGCG GTGACCGTCC  
 21151 GACACGTTGC GATACTGGTG TTTAGTGCTC CACTTAAACT CAGGCACAAC  
 CTGTGCAACG CTATGACCAC AAATCACGAG GTGAATTTGA GTCCGTGTTG  
 21201 CATCCGCGGC AGCTCGGTGA AGTTTTCACT CCACAGGCTG CGCACCATCA  
 GTAGGCGCCG TCGAGCCACT TCAAAAGTGA GGTGTCCGAC GCGTGSTAGT  
 21251 CCAACGCGTT TAGCAGGTCG GCGCCCGATA TCTTGAAGTC GCAGTTGGGG  
 GGTGCGCAA ATCGTCCAGC CCGCGGCTAT AGAAGTTCAG CGTCAACCCC  
 21301 CCTCCGCCCT GCGCGCGCGA GTTGCATAC ACAGGGTTGC AGCACTGGAA  
 GGAGGCGGGA CGCGCGCGCT CAACGCTATG TGTCCCAACG TCGTGACCTT  
 21351 CACTATCAGC GCCGGGTGGT GCACGCTGGC CAGCACGCTC TTGTCGGAGA  
 GTGATAGTCG CGGCCACCA CGTGCAGCCG GTCGTGCGAG AACAGCCTCT  
 21401 TCAGATCCGC GTCCAGGTCC TCCGCGTTGC TCAGGGCGAA CGGAGTCAAC  
 AGTCTAGGCG CAGGTCCAGG AGGCGCAACG AGTCCCGCTT GCCTCAGTTG  
 21451 TTTGTTAGCT GCCTTCCCAA AAAGGGCGCG TGCCCGAGCT TTGAGTTGCA  
 AAACCATCGA CGGAAGGGTT TTTCCCGCGC ACGGGTCCGA AACTCAACGT  
 21501 CTCGCACCGT AGTGGCATCA AAAGGTGACC GTGCCCCGTC TGGGCGTTAG  
 GAGCGTGGA TCACCGTAGT TTTCCACTGG CACGGGCCAG ACCCGCAATC  
 21551 GATACAGCGC CTGCATAAAA GCCTTGATCT GCTTAAAAGC CACCTGAGCC  
 CTATGTGCGG GACGTATTTT CGGAAGTAGA CGAATTTTCG GTGGACTCGG  
 21601 TTTGCGCCTT CAGAGAAGAA CATGCCGCAA GACTTGCCGG AAAACTGATT  
 AAACGCGGAA GTCTCTTCTT GTACGGCGTT CTGAACGGCC TTTTGACTAA  
 21651 GGCCGGACAG GCCGCGTCGT GCACGCAGCA CCTTGCGTCG GTGTTGGAGA  
 CCGGCCTGTC CGGCGCAGCA CGTGCCTCGT GGAACGCAGC CACAACCTCT  
 21701 TCTGCACCAC ATTTGCGCCC CACCGTTTCT TCACGATCTT GGCCTTGCTA  
 AGACGTGGTG TAAAGCCGGG GTGGCCAGA AGTGCTAGAA CCGGAACGAT

Figure 27 W

21751 GACTGCTCCT TCGCGCG CTGCCCCTTT TCGCTCGTCA CATTCATC  
 CTGACGAGGA AGTCGCGCGC GACGGGCAAA AGCGAGCAGT GTAGGTAAAG  
 21801 AATCACGTGC TCCTTATTTA TCATAATGCT TCCGTGTAGA CACTTAAGCT  
 TTAGTGCACG AGGAATAAAT AGTATTACGA AGGCACATCT GTGAATTCGA  
 21851 CGCCTTCGAT CTCAGCGCAG CGGTGCAGCC ACAACGCGCA GCCCCTGGGC  
 GCGGAAGCTA GAGTCGCGTC GCCACGTCGG TGTTGCGCGT CGGGCACCCG  
 21901 TCGTGATGCT TGTAGGTCAC CTCTGCAAAC GACTGCAGGT ACGCCCTGCAG  
 AGCACTACGA ACATCCAGTG GAGACGTTTG CTGACGTCCA TCGGACGCTC  
 21951 GAATCGCCCC ATCATCGTCA CAAAGGTCTT GTTGCTGGTG AAGGTCAGCT  
 CTTAGCGGGG TAGTAGCAGT GTTTCAGAA CAACGACCAC TTCCAGTCGA  
 22001 GCAACCCGCG GTGCTCCTCG TTCAGCCAGG TCTTGCCATC GGCCGCCAGA  
 CGTTGGGCGC CACGAGGAGC AAGTCGGTCC AGAACGTATG CCGGCGGTCT  
 22051 GCTTCCACTT GGTGAGGCAG TAGTTTGAAG TTCGCCCTTA GATCGTTATC  
 CGAAGGTGAA CCAGTCCGTC ATCAAACCTC AAGCGGAAAT CTAGCAATAG  
 22101 CACGTGGTAC TTGTCCATCA GCGCGCGCGC AGCCTCCATG CCCTTCTCCC  
 GTGCACCATG AACAGGTAGT CCGCGCGCGC TCGGAGGTAC GGGGAAGAGG  
 22151 ACGCAGACAC GATCGGCACA CTCAGCGGGT TCATCACCCT AATTTCACCT  
 TGCGTCTGTG CTAGCCGTGT GAGTCGCCCA AGTAGTGGCA TTAAAGTGAA  
 22201 TCCGCTTCGC TGGGCTCTTC CTCTTCTCTT TGCGTCCGCA TACCACGCGC  
 AGGCGAAGCG ACCCGAGAAG GAGAAGGAGA ACGCAGGCGT ATGGTGC GCG  
 22251 CACTGGGTCG TCTTCATTCA GCCGCCGCAC TGTGCGCTTA CCTCCTTTGC  
 GTGACCCAGC AGAAGTAAGT CGGCGGCGTG ACACGCGAAT GGAGGAAACG  
 22301 CATGCTTGAT TAGCACCAGT GGGTTGCTGA AACCACCAT TTGTAGCGCC  
 GTACGAATA ATCGTGGCCA CCAACGACT TTGGGTGGTA AACATCGCGG  
 22351 ACATCTTCTC TTTCTTCTC GCTGTCCACG ATTACCTCTG GTGATGGCGG  
 TGTAGAAGAG AAAGAAGGAG CGACAGGTGC TAATGGAGAC CACTACCGCC  
 22401 GCGCTCGGGC TTGGGAGAAG GCGCTTCTT TTTCTTCTTG GCGCAATGG  
 CGCGAGCCCG AACCTCTTC CCGCAAGAA AAAGAAGAAC CCGGTTACC  
 22451 CCAAATCCGC CGCCGAGGTC GATGGCCGCG GGCTGGGTGT GCGCGGCACC  
 GGTTTAGGCG GCGGCTCCAG CTACCGGCGC CCGACCCACA CGCGCGTGG  
 22501 AGCGCGTCTT GTGATGAGTC TTCCTCGTCC TCGGACTCGA TACGCCGCCT  
 TCGCGCAGAA CACTACTCAG AAGGAGCAGG AGCTGAGCT ATGCGGCGGA  
 22551 CATCCGCTTT TTTGGGGGCG CCCGGGGAGG CGCGGCGAC GGGGACGGG  
 GTAGCGGAAA AAACCCCGC GGGCCCTTC GCCGCGCTG CCCC TGCCCC  
 22601 ACGACACGTC CTCCATGGTT GGGGGACGTC GCGCGCACCC GCGTCCGCGC  
 TGCTGTGCAG GAGGTACCAA CCCCTGCGAG CGCGGCGTGG CGCAGGCGCG  
 22651 TCGGGGGTGG TTTGCGGCTG CTCTCTTCC CGACTGGCCA TTTCTTCTC  
 AGCCCCACC AAAGCGCGAC GAGGAGAAGG GCTGACCGGT AAAGGAAGAG

Figure 27X

22701 CTATAGGCAG AAGATCA TGGAGTCAGT CGAGAAGAAG GACAGCAG  
 GATATCCGTC TTTTCTAGT ACCTCAGTCA GCTCTTCTTC CTGTCCGATT  
 22751 CCGCCCCCTC TGAGTTCGCC ACCACCGCCT CCACCGATGC CGCCAACGCG  
 GCGGGGGGAG ACTCAAGCGG TGGTGGCGGA GGTGGCTACG GCGGTTGCGC  
 22801 CCTACCACCT TCCCCGTGCA GGCACCCCGG CTTGAGGAGG AGGAAGTGAT  
 GGATGGTGGA AGGGGCAGCT CCGTGGGGGC GAACTCCTCC TCCTTCACTA  
 22851 TATCGAGCAG GACCCAGGTT TTGTAAGCGA AGACGACGAG GACCGCTCAG  
 ATAGCTCGTC CTGGGTCCAA AACATTGCT TCTGCTGCTC CTGGCGAGTC  
 22901 TACCAACAGA GGATAAAAAG CAAGACCAGG ACAACGCAGA GGCAAACGAG  
 ATGGTTGTCT CCTATTTTTT GTTCTGGTCC TGTTGCGTCT CCGTTTGCTC  
 22951 GAACAAGTCG GCGGGGGGGA CGAAAGGCAT GCGCACTACC TAGATGTGGG  
 CTTGTTTACG CCGCCCCCTT GCTTTCGTA CCGCTGATGG ATCTACACCC  
 23001 AGACGACGTG CTGTTGAAGC ATCTGCAGCG CCAGTGCGCC ATTATCTGCG  
 TCTGCTGCAC GACAACTTCG TAGACGTGCG GGTACGCGG TAATAGACGC  
 23051 ACGCGTTGCA AGAGCGCAGC GATGTGCCCC TCGCCATAGC GGATGTCAGC  
 TGCGCAACGT TCTCGCGTCG CTACACGGGG AGCGGTATCG CCTACAGTCG  
 23101 CTGCGCTACG AACGCCACCT ATTCTCACCG CGCGTACCCC CCAAACGCCA  
 GAACGGATGC TTGCGGTGGA TAAGAGTGGC GCGCATGGGG GGTGCGGT  
 23151 AGAAAACGCG ACATGCGAGC CCAACCCGCG CCTCAACTTC TACCCCGTAT  
 TCTTTTGCCG TGTACGCTCG GGTGGGCGC GGAGTTGAAG ATGGGGCATA  
 23201 TTGCCGTGCC AGAGGTGCTT GCCACCTATC ACATCTTTTT CCAAAACTGC  
 AACGGCACGG TCTCCACGAA CGGTGGATAG TGTAGAAAAA GGTGTTGACG  
 23251 AAGATACCCC TATCTGCGG TGCCAACCGC AGCCGAGCGG ACAAGCAGCT  
 TTCTATGGGG ATAGGACGGC ACGGTTGGCG TCGGCTCGCC GTTCGTCGA  
 23301 GGCTTGCAG CAGGGCGCTG TCATACCTGA TATCGCCTCG CTCAACGAAG  
 CCGGAACGCC GTCCCGCGAC AGTATGGACT ATAGCGGAGC GAGTTGCTTC  
 23351 TGCCAAAAAT CTTTGAGGGT CTTGGACGCG ACGAGAAGCG CGCGGCAAAC  
 ACGGTTTTTA GAACTCCCA GAACCTGCGC TGCTCTTCGC GCGCCGTTTG  
 23401 GCTCTGCAAC AGGAAAAACAG CGAAAATGAA AGTCACTCTG GAGTGTGGT  
 CGAGACGTTG TCCTTTTGTC GCTTTTACTT TCAGTGAGAC CTCACAACCA  
 23451 GGAACTCGAG GGTGACAACG CGCGCCTAGC CGTACTAAAA CGCAGCATCG  
 CCTTGAGCTC CCACTGTTGC GCGCGGATCG GCATGATTTT GCGTCGTAGC  
 23501 AGGTCACCCA CTTTGCTTAC CCGGCCTTA ACCTACCCCC CAAGGTCATG  
 TCCAGTGGGT GAAACGGATG GGCCGTGAAT TGGATGGGGG GTTCCAGTAC  
 23551 AGCACAGTCA TGAGTGAGCT GATCGTGCGC CGTGCGCAGC CCCTGGAGAG  
 TCGTGTCAGT ACTCACTCGA CTAGCACGCG GCACGCGTCG GGSACCTCTC  
 23601 GGATGCAAAAT TTGCAAGAAC AAACAGAGGA GGGCCTACCC GCAGTTGGCG  
 CCTACGTTTA AACGTTCTTG TTTGTCTCCT CCCGGATGGG CGTCAACCGC

Figure 27 Y

23651 ACGAGCAGCT ACGCTGG CTTCAAACGC GCGAGCCTGC CGACTTGG  
TGCTCGTCTGA TCGCGCGACC GAAGTTTGCG CGCTCGGACG GCTGAACCTC

23701 GAGCGACGCA AACTAATGAT GGCCGCAGTG CTCGTTACCG TGGAGCTTGA  
CTCGCTGCGT TTGATTACTA CCGGCCTCAC GAGCAATGGC ACCTCGAACT

23751 GTGCATGCAG CGGTTCTTTG CTGACCCGGA GATGCAGCGC AAGCTAGAGG  
CACGTACGTC GCCAAGAAAC GACTGGGCCT CTACGTCGCG TTCGATCTCC

23801 AAACATTGCA CTACACCTTT CGACAGGGCT ACGTACGCCA GGCTTGCAAG  
TTTGTAACGT GATGTGAAA GCTGTCCCGA TGCATGCGGT CCGGACGTTT

23851 ATCTCCAACG TGGAGCTCTG CAACCTGGTC TCCTACCTTG GAATTTTGCA  
TAGAGGTTGC ACCTCGAGAC GTTGACCAG AGGATGGAAC CTTAAAACGT

23901 CGAAAACCGC CTTGGGCAAA ACGTGCTTCA TTCCACGCTC AAGGGCGAGG  
GCTTTTGCG GAACCCGTTT TGCACGAAGT AAGGTGCGAG TTCCCGCTCC

23951 CGCGCCGCGA CTACGTCCGC GACTGCGTTT ACTTATTTCT ATGCTACACC  
GCGCGGCGCT GATGCAGCG CTGACGCAA TGAATAAAGA TACGATGTGG

24001 TGGCAGACGG CCATGGGCGT TTGGCAGCAG TGCTTGAGG AGTGCAACCT  
ACCGTCTGCC GGTACCCGCA AACCGTCGTC ACGAACCTCC TCACGTTGGA

24051 CAAGGAGCTG CAGAACTGC TAAAGCAAAA CTTGAAGGAC CTATGGACGG  
GTTCTCTGAC GTCTTTGACG ATTTCGTTTT GAACCTCTG GATACCTGCC

24101 CCTTCAACGA GCGCTCCGTG GCCGCGCACC TGGCGGACAT CATTTTCCCC  
GGAAGTTGCT CCGGAGGCAC CGCGCGGTGG ACCGCTGTA GTAAAAGGGG

24151 GAACGCCTGC TTAACCCT GCAACAGGGT CTGCCAGACT TCACCAGTCA  
CTTGCGGACG AATTTTGGA CGTTGTCCCA GACGGTCTGA AGTGGTCAGT

24201 AAGCATGTTG CAGAACTTA GGAACCTTAT CCTAGAGCGC TCAGGAATCT  
TTCGTACAAC GTCTTGAAAT CCTTGAAATA GGATCTCGCG AGTCCTTAGA

24251 TGCCCCCACC CTGCTGTGCA CTTCTAGCG ACTTTGTGCC CATTAGTAC  
ACGGGCGGTG GACGACACGT GAAGGATCGC TGAACACGG GTAATTCATG

24301 CGCGAATGCC CTCCGCCGCT TTGGGGCCAC TGCTACCTTC TGCAGCTAGC  
GCGCTTACGG GAGGCGGCGA AACCCCGGTG ACGATGGAAG ACGTCGATCG

24351 CAACTACCTT GCCTACCACT CTGACATAAT GGAAGACGTG AGCGGTGACG  
GTTGATGGAA CGGATGGTGA GACTGTATTA CCTTCTGCAC TCGCCACTGC

24401 GTCTACTGGA GTGTCACTGT CGCTGCAACC TATGCACCCC GCACCGCTCC  
CAGATGACCT CACAGTGACA GCGACGTTGG ATACGTGGGG CGTGGCGAGG

24451 CTGGTTTGCA ATTGCGAGCT GCTTAACGAA AGTCAAATTA TCGGTACCTT  
GACCAAACGT TAAGCGTCGA CGAATTGCTT TCAGTTTAAT AGCCATGGAA

24501 TGAGCTGCAG GGTCCCTCGC CTGACGAAA GTCCGCGGCT CCGGGGTTGA  
ACTCGACGTC CCAGGGAGCG GACTGCTTTT CAGGCGCCGA GGCCCAACT

24551 AACTCACTCC GGGGCTGTGG ACGTCGGCTT ACCTTCGCAA ATTTGTACCT  
TTGAGTGAGG CCCCACACC TGCAGCCGAA TGAAGCGTT TAAACATGGA

Figure 272



24601 GAGGACTACC AC~~CC~~CCACGA GATTAGGTTC TACGAAGACC AATCCCG~~CC~~  
 CTCTGATGG TCGGGGTGCT CTAATCCAAG ATGCTTCTGG TTAGGGCGGG  
 24651 GCCTAATGCG GAGCTTACCG CCTGCGTCAT TACCCAGGGC CACATTCTTG  
 CGGATTACGC CTCGAATGGC GGACGCAGTA ATGGGTCCCG GTGTAAGAAG  
 24701 GCCAATTGCA AGCCATCAAC AAAGCCCGCC AAGAGTTTCT GCTACGAAAG  
 CGGTAAACGT TCGGTAGTTG TTTCGGGCGG TTCTCAAAGA CGATGCTTTC  
 24751 GGACGGGGGG TTTACTTGGA CCCCAGTCC GCGGAGGAGC TCAACCCAAT  
 CCTGCCCCC AAATGAACCT GGGGGTCAGG CCGCTCCTCG AGTTGGGTTA  
 24801 CCCCCGCGG CCGCAGCCCT ATCAGCAGCA GCGCGGGCC CTTGCTTCCC  
 GGGGGCGGGG GGCGTCGGGA TAGTCGTCGT CGCGCGCCGG GAACGAAGGG  
 24851 AGGATGGCAC CAAAAAGAA GCTGCAGCTG CCGCCGCCAC CCACGGACGA  
 TCCTACCGTG GGT~~TTTT~~CTT CGACGTCGAC GCGGCGGTG GGTGCTGCT  
 24901 GGAGGAATAC TGGGACAGTC AGGCAGAGGA GGT~~TTTT~~GAC GAGGAGGAGG  
 CTTCTTATG ACCCTGTCAG TCCGTCTCCT CAAAAACCTG CTCTCTCTCC  
 24951 AGGACATGAT GGAAGACTGG GAGAGCCTAG ACGAGGAAGC TTCCGAGGTC  
 TCCTGTACTA CCTTCTGACC CTCTCGGATC TGCTCCTTCG AAGGCTCCAG  
 25001 GAAGAGGTGT CAGACGAAAC ACCGTCACCC TCGGTCGCAT TCCCTCGCC  
 CTTCTCCACA GTCTGCTTTG TGGCAGTGGG AGCCAGCGTA AGGGGAGCGG  
 25051 GCGCCCCCAG AAATCGGCAA CCGGTTCCAG CATGGCTACA ACCTCCGCTC  
 CCGCGGGGTC TTTAGCCGTT GGCCAAGGTC GTACCGATGT TGGAGGCGAG  
 25101 CTCAGGCGCC GCCGGCACTG CCGGTTCCGC GACCCAACCG TAGATGGGAC  
 GAGTCCGCGG CGGCCGTGAC GGGCAAGCGG CTGGGTTGGC ATCTACCCTG  
 25151 ACCACTGGAA CCAGGGCCGG TAAGTCCAAG CAGCCGCCGC CGTTAGCCCA  
 TGGTGACCTT GGTCCCGGCC ATTCAAGTTC GTCGGCGGCG GCAATCGGGT  
 25201 AGAGCAACAA CAGCGCCAAG GCTACCGCTC ATGGCGCGGG CACAAGAACG  
 TCTCGTTGTT GTCGCGGTTT CGATGGCGAG TACCGCGCCC GTGTCTTGC  
 25251 CCATAGTTGC TTGCTTGCAA GACTGTGGGG GCAACATCTC CTTGCCCCGC  
 GGTATCAACG AACGAACGTT CTGACACCCC CGTTGTAGAG GAAGCGGGCG  
 25301 CGCTTCTTTC TCTACCATCA CGGCGTGGCC TTCCCCCGTA ACATCCTGCA  
 GCGAAAGAAG AGATGGTAGT GCCGCACCGG AAGGGGGCAT TGTAGGACGT  
 25351 TTACTACCGT CATCTCTACA GCCCATACTG CACCGGCGGC AGCGGCAGCA  
 AATGATGGCA GTAGAGATGT CCGGTATGAC GTGGCCGCGG TCGCCGTCGT  
 25401 ACAGCAGCGG CCACACAGAA GCAAAGCGGA CCGGATAGCA AGACTCTGAC  
 TGTCGTCGCC GGTGTGTCTT CGTTTCCGCT GGCTATCGT TCTGAGACTG  
 25451 AAAGCCCAAG AAATCCACAG CGGCGGCAGC AGCAGGAGGA GGAGCGCTGC  
 TTTCCGGTTC TTTAGGTGTC GCCGCCGTG TCGTCCTCCT CCTCGCGACG  
 25501 GTCTGGCGCC CAACGAACCC GTATCGACCC GCGAGCTTAG AAACAGGATT  
 CAGACCGCGG GTTGCTTGGG CATAGCTGGG CGCTCGAATC TTTGTCTTAA

Figure 27. AA

25551 TTTCCCACTC TCTTGCTAT ATTCAACAG AGCAGGGGCC AAGAACA  
 AAAGGGTGAG ACATACGATA TAAAGTTGTC TCGTCCCCGG TTCTTGTTCT

25601 GCTGAAAATA AAAACAGGT CTCTGCGATC CCTCACCCGC AGCTGCCTGT  
 CGACTTTTAT TTTTGTCCA GAGACGCTAG GGAGTGGGCG TCGACGGACA

25651 ATCACAAAAG CGAAGATCAG CTTGCGCGCA CGCTGGAAGA CGCGGAGGCT  
 TAGTGTTTTT GCTTCTAGTC GAAGCCGCGT GCGACCTTCT GCGCCTCCGA

25701 CTCTTCAGTA AATACTGCGC GCTGACTCTT AAGGACTAGT TTCGCGCCCT  
 GAGAAGTCAT TTATGACGCG CGACTGAGAA TTCTTGATCA AAGCGCGGGA

25751 TTCTCAAATT TAAGCGCGAA AACTACGTCA TCTCCAGCGG CCACACCCGG  
 AAGAGTTTAA ATTGCGGCTT TTGATGCACT AGAGGTCGCC GGTGTGGGCC

25801 CGCCAGCACC TGTGTGTCAGC GCCATTATGA GCAAGGAAAT TCCCACGCCC  
 GCGGTGCTGG ACAACAGTCG CGGTAATACT CGTTCCTTTA AGGGTGCGGG

25851 TACATGTGGA GTTACCAGCC ACAAATGGGA CTTGCGGCTG GAGCTGCCCCA  
 ATGTACACCT CAATGGTCGG TGTTTACCCT GAACGCCGAC CTCGACGGGT

25901 AGACTACTCA ACCCGAATAA ACTACATGAG CGCGGGACCC CACATGATAT  
 TCTGATGAGT TGGGCTTATT TGATGTACTC GCGCCCTGGG GTGTACTATA

25951 CCCGGGTCAA CGGAATACGC GCCCACCAGAA ACCGAATTCT CCTGGAACAG  
 GGGCCAGTT GCCTTATGCG CGGGTGGCTT TGGCTTAAGA GGACCTTGTC

26001 GCGGCTATTA CCACCACACC TCGTAATAAC CTTAATCCCC GTAGTTGGCC  
 CGCCGATAAT GGTGGTGTGG AGCATTATG GAATTAGGGG CATCAACCGG

26051 CGCTGCCCTG GTGTACCAGG AAAGTCCCGC TCCCACCACT GTGGTACTTC  
 GCGACGGGAC CACATGGTCC TTTCAGGGCG AGGGTGGTGA CACCATGAAG

26101 CCAGAGACGC CCAGGCCGAA GTTCAGATGA CTAACTCAGG GGCGCAGCTT  
 GGTCCTGCG GGTCCGGCTT CAAGTCTACT GATTGAGTCC CCGCGTCGAA

26151 GCGGGCGGCT TTCGTACAG GGTGCGGTCG CCCGGGCAGG GTATAACTCA  
 CGCCCGCCGA AAGCAGTGT CACGCCAGC GGGCCCGTCC CATATTGAGT

26201 CCTGACAATC AGAGGGCGAG GTATTAGCT CAACGACGAG TCGGTGAGCT  
 GGACTGTTAG TCTCCCGCTC CATAAGTCGA GTTGCTGCTC AGCCACTCGA

26251 CCTCGCTTGG TCTCCGTCCG GACGGGACAT TTCAGATCGG CGGCGCCGGC  
 GGAGCGAACC AGAGGCAGGC CTGCCCCTGA AAGTCTAGCC GCCCGGCCG

26301 CGCTCTTCAT TCACGCCTCG TCAGGCAATC CTAACTCTGC AGACCTCGTC  
 CCGAGAACTA AGTGCGGAGC AGTCCGTTAG GATTGAGACG TCTGGAGCAG

26351 CTCTGAGCCG CGCTCTGGAG GCATTGGAAC TCTGCAATTT ATTGAGGAGT  
 GAGACTCGGC GCGAGACCTC CGTAACCTTG AGACGTTAAA TAACTCCTCA

26401 TTGTGCCATC GGTCTACTTT AACCCTTCT CGGGACCTCC CGGCCACTAT  
 AACACGGTAG CCAGATGAAA TTGGGGAAGA GCCCTGGAGG GCCGGTGATA

26451 CCGGATCAAT TTATTCCTAA CTTTGACGCG GTAAAGGACT CGGCGGACGG  
 GGCTAGTTA AATAAGGATT GAAACTGCGC CATTTCTGA GCCGCTGCC

Figure 27 AB

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26501 CTACGACTGA A TAAAGTG GAGAGGCAGA GCAACTGCCG CTGAAA C
      GATGCTGACT TACAATTCAC CTCTCCGTCT CGTTGACGCG GACTTTGTGG

26551 TGGTCCACTG TCGCCGCCAC AAGTGCTTTG CCCGCGACTC CGGTGAGTTT
      ACCAGGTGAC AGCGGCGGTG TTCACGAAAC GGGCGCTGAG GCCACTCAA

26601 TGCTACTTTG AATTGCCCGA GGATCATATC GAGGGCCCGG CGCACGGCGT
      ACGATGAAAC TTAACGGGCT CCTAGTATAG CTCCCGGGCC GCGTGCCGCA

26651 CCGGCTTACC GCCCAGGGAG AGCTTGCCCG TAGCCTGATT CGGGAGTTTA
      GGCCGAATGG CGGTCCCTC TCGAACGGGC ATCGGACTAA GCCCTCAAAT

26701 CCCAGCGCCC CCTGCTAGTT GAGCGGGACA GGGGACCCTG TGTCTCACT
      GGGTCGCGGG GGACGATCAA CTCGCCCTGT CCCCTGGGAC ACAAGAGTGA

26751 GTGATTTGCA ACTGTCCTAA CCCTGGATTA CATCAAGATC TTTGTTGCCA
      CACTAAACGT TGACAGGATT GGGACCTAAT GTAGTTCTAG AAACAACGGT

26801 TCTCTGTGCT GAGTATAATA AATACAGAAA TTAAAATATA CTGGGGCTCC
      AGAGACACGA CTCATATTAT TTATGTCTTT AATTTTATAT GACCCCAGG

26851 TATCGCCATC CTGTAAACGC CACCGTCTTC ACCCGCCCAA GCAAACCAAG
      ATAGCGGTAG GACATTGCG GTGGCAGAAG TGGGCGGGTT CTTTGGTTC

26901 GCGAACCTTA CCTGCTACTT TTAACATCTC TCCCTCTGTG ATTTACAACA
      CGCTTGGAAT GGACCATGAA AATTGTAGAG AGGGAGACAC TAAATGTTGT

26951 GTTTC AACCC AGACGGAGTG AGTCTACGAG AGAACCTCTC CGAGCTCAGC
      CAAAGTTGGG TCTGCCTCAC TCAGATGCTC TCTTGAGAG GCTCGAGTCG

27001 TACTCCATCA GAAAAACAC CACCCTCCTT ACCTGCCGGG AACGTACGAG
      ATGAGGTAGT CTTTTTGTG GTGGGAGGAA TGGACGGCCC TTGCATGCTC

27051 TGCCTCACCG GCCGCTGCAC CACACCTACC GCCTGACCGT AAACCAGACT
      ACGCAGTGGC CGGCGACGTG GTGTGGATGG CGGACTGGCA TTTGGTCTGA

27101 TTTTCCGGAC AGACCTCAAT AACTCTGTTT ACCAGAACAG GAGGTGAGCT
      AAAAGGCCTG TCTGGAGTTA TTGAGACAAA TGGTCTTGTC CTCCACTCGA

27151 TAGAAAACCC TTAGGGTATT AGCCCAAAGG CGCAGCTACT GTGGGGTTTA
      ATCTTTTGGG AATCCCATAA TCCGGTTTCC GCGTCGATGA CACCCCAAAT

27201 TGAACAATTC AAGCAACTCT ACGGGCTATT CTAATTCAGG TTTCTCTAGA
      ACTTGTTAAG TTCGTTGAGA TGCCCGATAA GATTAAGTCC AAAGAGATCT

27251 ATCGGGGTTG GGGTTATTCT CTGTCTTGTG ATTCTCTTTA TTCTTATACT
      TAGCCCCAAC CCCAATAAGA GACAGAACAC TAAGAGAAAT AAGAATATGA

27301 AACGCTTCTC TGCCTAAGGC TCGCCGCCCTG CTGTGTGCAC ATTTGCATTT
      TTGCGAAGAG ACGGATTCCG AGCGGCGGAC GACACACGTG TAAACGTAAA

27351 ATTGTGAGCT TTTTAAACGC TGGGGTCGCC ACCCAAGATG ATTAGGTACA
      TAACAGTCGA AAAATTGCG ACCCCAGCGG TGGGTTCTAC TAATCCATGT

27401 TAATCCTAGG TTTACTCACC CTGCGTCAG CCCACGGTAC CACCCAAAAG
      ATTAGGATCC AAATGAGTGG GAACGCAGTC GGGTGCCATG GTGGGTTTTT

```

Figure 27 AC

27451 GTGGATTTTA A G C C C A G C CTGTAATGTT ACATTGCGAG CTGAAG G A  
 CACCTAAAAT TCCTCGGTG GACATTACAA TGTAAGCGTC GACTTCGATT  
 27501 TGAGTGCACC ACTCTTATAA AATGCACCAC AGAACATGAA AAGCTGCTTA  
 ACTCACGTGG TGAGAATATT TTACGTGGTG TCTTGACTT TTCGACGAAT  
 27551 TTCGCCACAA AAACAAAATT GGCAAGTATG CTGTTTATGC TATTTGGCAG  
 AAGCGGTGTT TTTGTTTTAA CCGTTCATAC GACAAATACG ATAAACCGTC  
 27601 CCAGGTGACA CTACAGAGTA TAATGTTTCA GTTTTCCAGG GTAAAAGTCA  
 GGTCCACTGT GATGCTCAT ATTACAATGT CAAAAGGTCC CATTTTCAGT  
 27651 TAAACTTTT ATGTATACTT TTCCATTTTA TGAAATGTGC GACATTACCA  
 ATTTTGAAA TACATATGAA AAGGTAAAAT ACTTTACACG CTGTAATGGT  
 27701 TGTACATGAG CAAACAGTAT AAGTTGTGGC CCCCACAAA TTGTGTGGAA  
 ACATGTACTC GTTGTGCATA TTCAACACCG GGGGTGTTTT AACACACCTT  
 27751 AACACTGGCA CTTTCTGCTG CACTGCTATG CTAATTACAG TGCTCGCTTT  
 TTGTGACCGT GAAAGACGAC GTGACGATAC GATTAATGTC ACGAGCGAAA  
 27801 GGTCTGTACC CTACTCTATA TTAAATACAA AAGCAGACGC AGCTTTATTG  
 CCAGACATGG GATGAGATAT AATTTATGTT TTCGTCTGCG TCGAAATAAC  
 27851 AGGAAAAGAA AATGCCTTAA TTTACTAAGT TACAAAGCTA ATGTCACCAC  
 TCCTTTTCTT TTACGGAATT AAATGATTCA ATGTTTCGAT TACAGTGGTG  
 27901 TAACTGCTTT ACTCGCTGCT TGCAAAACAA ATTCAAAAAG TTAGCATTAT  
 ATTGACGAAA TGAGCGACGA ACGTTTTGTT TAAGTTTTTC AATCGTAATA  
 27951 AATTAGAATA GGATTTAAAC CCCCCGGTCA TTTCTGCTC AATACCATT  
 TTAATCTTAT CCTAAATTG GGGGGCCAGT AAAGGACGAG TTATGGTAAG  
 28001 CCCTGAACAA TTGACTCTAT GTGGGATATG CTCCAGCGCT ACAACCTTGA  
 GGGACTTGTT AACTGAGATA CACCCTATAC GAGGTGCGA TGTGGAAC  
 28051 AGTCAGGCTT CCTGGATGTC AGCATCTGAC TTTGGCCAGC ACCTGTCCCG  
 TCAGTCCGAA GGACCTACAG TCGTAGACTG AAACCGGTCG TGGACAGGGC  
 28101 CGGATTTGTT CCAGTCCAAC TACAGCGACC CACCCTAACA GAGATGACCA  
 GCCTAAACAA GGTGAGTTG ATGTCGCTGG GTGGGATTGT CTCTACTGGT  
 28151 ACACAACCAA CGCGCCGCC GCTACCGGAC TTACATCTAC CACAAATACA  
 TGTGTTGGTT GCGCCGGCGG CGATGGCCTG AATGTAGATG GTGTTTATGT  
 28201 CCCCAGTTT CTGCCTTTGT CAATAACTGG GATAACTTGG GCATGTGGTG  
 GGGGTTCAAA GACGGAAACA GTTATTGACC CTATTGAACC CGTACACCAC  
 28251 GTTCTCCATA GCGCTTATGT TTGTATGCCT TATTATTATG TGGCTCATCT  
 CAAGAGGTAT CGCGAATACA AACATACGGA ATAATAATAC ACCGAGTAGA  
 28301 GCTGCCTAAA GCGCAAACGC GCCCGACCAC CCATCTATAG TCCCATCATT  
 CGACGGATTT CGCGTTTGGC GGGGCTGGTG GGTAGATATC AGGCTAGTAA  
 28351 GTGCTACACC CAAACAATGA TGAATCCAT AGATTGGACG GACTGAAACA  
 CACGATGTGG GTTGTACT ACCTTAGGTA TCTAACCTGC CTGACTTTGT

Figure 27A D

28401 CATGTTCTTT TTTACAG TATGATTAAA TGAGACATGA TTCCTCTTT  
 GTACAAGAAA AGAGAATGTC ATACTAATTT ACTCTGTACT AAGGAGCTCA  
 28451 TTTTATATTA CTGACCCTTG TTGCGCTTTT TTGTGCGTGC TCCACATTGG  
 AAAATATAAT GACTGGGAAC AACGCGAAAA AACACGCACG AGGTGTAACC  
 28501 CTGCGGTTTC TCACATCGAA GTAGACTGCA TTCCAGCCTT CACAGTCTAT  
 GACGCCAAAG AGTGTAGCTT CATCTGACGT AAGGTCGGAA GTGTCAGATA  
 28551 TTGCTTTACG GATTGTGCAC CCTCACGCTC ATCTGCAGCC TCATCACTGT  
 AACGAAATGC CTAACAGTG GGAGTGCAG TAGACGTCGG AGTAGTGACA  
 28601 GGTTCATCGCC TTTATCCAGT GCATTGACTG GGTCTGTGTG CGCTTTGCAT  
 CCAGTAGCGG AAATAGGTCA CGTAAC TGAC CCAGACACAC GCGAAACGTA  
 28651 ATCTCAGACA CCATCCCCAG TACAGGGACA GGACTATAGC TGAGCTTCTT  
 TAGAGTCTGT GGTAGGGGTC ATGTCCCTGT CCTGATATCG ACTCGAAGAA  
 28701 AGAATTCTTT AATTATGAAA TTTACTGTGA CTTTCTGTCT GATTATTTGC  
 TCTTAAGAAA TTAATACTTT AAATGACACT GAAAAGACGA CTAATAAACG  
 28751 ACCCTATCTG CGTTTTGTTC CCCGACCTCC AAGCCTCAAA GACATATATC  
 TGGGATAGAC GCAAAACAAG GGGCTGGAGG TTCGGAGTTT CTGTATATAG  
 28801 ATGCAGATTC ACTCGTATAT GGAATATTCC AAGTTGCTAC AATGAAAAAA  
 TACGTCTAAG TGAGCATATA CCTTATAAGG TTCAACGATG TTACTTTTTT  
 28851 GCGATCTTTC CGAAGCCTGG TTATATGCAA TCATCTCTGT TATGGTGTTC  
 CGCTAGAAAG GCTTCGGACC AATATACGTT AGTAGAGACA ATACCACAAG  
 28901 TGCAGTACCA TCTTAGCCCT AGCTATATAT CCCTACCTTG ACATTGGCTG  
 ACGTCATGGT AGAATCGGGA TCGATATATA GGGATGGAAC TGTAACCGAC  
 28951 GAACGCAATA GATGCCATGA ACCACCCAAC TTTCCCCGCG CCCGCTATGC  
 CTTGCGTTAT CTACGGTACT TGGTGGGTTG AAAGGGGCGC GGGCGATACG  
 29001 TTCCACTGCA ACAAGTTGTT GCCGGCGGCT TTGTCCCAGC CAATCAGCCT  
 AAGGTGACGT TGTTCAACAA CGGCCGCCGA AACAGGGTCG GTTAGTCGGA  
 29051 CGCCACCTT CCCCCACCCC CACTGAAATC AGCTACTTTA ATCTAACAGG  
 CGGGGTGGA GAGGGTGGGG GTGACTTTAG TCGATGAAAT TAGATTGTCC  
 29101 AGGAGATGAC TGACACCCTA GATCTAGAAA TGGACGGAAT TATTACAGAG  
 TCCTCTACTG ACTGTGGGAT CTAGATCTTT ACCTGCCTTA ATAATGTCTC  
 29151 CAGCGCCTGC TAGAAAGACG CAGGGCAGCG GCCGAGCAAC AGCGCATGAA  
 GTCGCGGACG ATCTTTCTGC GTCCCGTCGC CGGCTCGTTG TCGCGTACTT  
 29201 TCAAGAGCTC CAAGACATGG TTAAC TTGCA CCAGTGCAAA AGGGGTATCT  
 AGTTCTCGAG GTTCTGTACC AATTGAACGT GGTACGTTT TCCCCATAGA  
 29251 TTTGTCTCGT AAAGCAGGCC AAAGTCACCT ACGACAGTAA TACCACCGGA  
 AAACAGAGCA TTTCGTCCGG TTTCAGTGGA TGCTGTCAAT ATGGTGGCCT  
 29301 CACCGCCTTA GCTACAAGTT GCCAACCAAG CGTCAGAAAT TGGTGGTCAT  
 GTGGCGGAAT CGATGTTCAA CGGTGGTTC GCAGTCTTTA ACCACCAGTA

Figure 27 A E

29351 GGTGGGAGAA A~~CC~~CCATTA CCATAACTCA GCACTCGGTA GAAACC~~CG~~  
 CCACCCCTCTT TTCGGGTAAT GGTATTGAGT CGTGAGCCAT CTTTGGCTTC

29401 GCTGCATTCA CTCACCTTGT CAAGGACCTG AGGATCTCTG CACCCTTATT  
 CGACGTAAGT GAGTGAACA GTTCTGGAC TCCTAGAGAC GTGGGAATAA

29451 AAGACCCGTG GCGGTCTCAA AGATCTTATT CCCTTTAACT AATAAAAAA  
 TTCTGGGACA CGCCAGAGTT TCTAGAATAA GGGAAATTGA TTATTTTTTT

29501 AATAATAAAG CATCACTTAC TTAAAATCAG TTAGCAAATT TCTGTCCAGT  
 TTATTATTTC GTAGTGAATG AATTTTAGTC AATCGTTTAA AGACAGGTCA

29551 TTATTTCAGCA GCACCTCCTT GCCCTCCTCC CAGCTCTGGT ATTGCAGCTT  
 AATAAGTCGT CGTGGAGGAA CGGGAGGAGG GTCGAGACCA TAACGTCGAA

29601 CCTCCTGGCT GCAAACCTTC TCCACAATCT AAATGGAATG TCAGTTTCCT  
 GGAGGACCGA CGTTTGAAG AGGTGTTAGA TTTACCTTAC AGTCAAAGGA

29651 CCTGTTCTCG TCCATCCGCA CCCACTATCT TCATGTTGTT GCAGATGAAG  
 GGACAAGGAC AGGTAGGCGT GGGTGATAGA AGTACAACAA CGTCTACTTC

29701 CGCGCAAGAC CGTCTGAAGA TACCTTCAAC CCCGTGTATC CATATGACAC  
 GCGCGTTCTG GCAGACTTCT ATGGAAGTTG GGGCACATAG GTATACTGTG

29751 GGAAACCGGT CCTCCAAC TGCCCTTTCT TACTCCTCCC TTTGTATCCC  
 CCTTTGGCCA GGAGGTTGAC ACGGAAAAGA ATGAGGAGGG AAACATAGGG

29801 CCAATGGGTT TCAAGAGAGT CCCCTGGGG TACTCTCTTT GCGCCTATCC  
 GGTTACCCAA AGTTCTCTCA GGGGGACCCC ATGAGAGAAA CGCGGATAGG

29851 GAACCTCTAG TTACCTCCAA TGGCATGCTT GCGCTCAAAA TGGGCAACGG  
 CTTGGAGATC AATGGAGGTT ACCGTACGAA CGCGAGTTT ACCCGTTGCC

29901 CCTCTCTCTG GACGAGGCCG GCAACCTTAC CTCCAAAAT GTAACCACTG  
 GGAGAGAGAC CTGCTCCGGC CGTTGGAATG GAGGGTTTTA CATTGGTGAC

29951 TGAGCCCAACC TCTCAAAAAA ACCAAGTCAA ACATAAACCT GGAAATATCT  
 ACTCGGGTGG AGAGTTTTTT TGGTTCAGTT TGTATTTGGA CCTTTATAGA

30001 GCACCCCTCA CAGTTACCTC AGAAGCCCTA ACTGTGGCTG CCGCCGCACC  
 CGTGGGGAGT GTCAATGGAG TCTTCGGGAT TGACACCGAC GCGGGCGTGG

30051 TCTAATGGTC GCGGGCAACA CACTCACCAT GCAATCACAG GCCCCGCTAA  
 AGATTACCAG CGCCCCTTGT GTGAGTGGTA CGTTAGTGTC CGGGGCGATT

30101 CCGTGACGCA CTCCAAACTT AGCATTGCCA CCCAAGGACC CCTCACAGTG  
 GGCACGTGCT GAGGTTTGAA TCGTAACGGT GGGTTCCTGG GGAGTGTCAC

30151 TCAGAAGGAA AGCTAGCCCT GCAAACATCA GGCCCCCTCA CCACCACCGA  
 AGTCTTCCTT TCGATCGGGA CGTTTGTAGT CCGGGGAGT GGTGGTGGCT

30201 TAGCAGTACC CTTACTATCA CTGCCTCACC CCCTCTAACT ACTGCCACTG  
 ATCGTCATGG GAATGATAGT GACGGAGTGG GGGAGATTGA TGACGGTGAC

30251 GTAGCTTGGG CATTGACTTG AAAGAGCCCA TTTATACACA AAATGGAAAA  
 CATCGAACCC GTAACGAAC TTTCTCGGGT AAATATGTGT TTTACCTTTT

Figure 27 AF

30301 CTAGGACTAA ACGGGG TCCTTTGCAT GTAACAGACG ACCTAAAC  
 GATCCTGATT TCGCCCCG AGGAAACGTA CATGTCTGC TGGATTCTG  
 30351 TTTGACCGTA GCAACTGGTC CAGGTGTGAC TATTAATAAT ACTTCCTTGC  
 AAACCTGGCAT CGTTGACCAG GTCCACACTG ATAATTATTA TGAAGGAACG  
 30401 AAACCTAAAGT TACTGGAGCC TTGGGTTTGT ATTCACAAGG CAATATGCAA  
 TTTGATTTC AATGACCTCGG AACCCAAAC TAAGTGTTC GTTATACGTT  
 30451 CTTAATGTAG CAGGAGGACT AAGGATTGAT TCTCAAAACA GACGCCTTAT  
 GAATTACATC GTCCCTCTGA TTCCTAATA AGAGTTTTGT CTGCGGAATA  
 30501 ACTTGATGTT AGTTATCCGT TTGATGCTCA AAACCAACTA AATCTAAGAC  
 TGAACACAA TCAATAGGCA AACTACGAGT TTTGGTTGAT TTAGATTCTG  
 30551 TAGGACAGGG CCCTCTTTTT ATAAACTCAG CCCACAACCT GGATATTAAC  
 ATCCTGTCCC GGGAGAAAAA TATTTGAGTC GGGTGTGAA CCTATAATTG  
 30601 TACAACAAAG GCCTTTACTT GTTTACAGCT TCAACAATT CCAAAAAGCT  
 ATGTTGTTTC CGGAAATGAA CAAATGTGCA AGTTTGTTAA GGTTTTTCGA  
 30651 TGAGGTTAAT CTAAGCACTG CCAAGGGGTT GATGTTTGAC GCTACAGCCA  
 ACTCCAATTG GATTCTGTGAC GGTTCCTCAA CTACAACTG CGATGTCGGT  
 30701 TAGCCATTAA TGCAGGAGAT GGGCTTGAAT TTGGTTCACC TAATGCACCA  
 ATCGGTAATT ACGTCTCTA CCCGAACCTA AACCAAGTGG ATTACGTGGT  
 30751 AACACAAATC CCCTCAAAAC AAAAATTGGC CATGGCCTAG AATTGATTTC  
 TTGTGTTTAG GGGAGTTTTG TTTTAAACCG GTACCGGATC TTAACATAAG  
 30801 AAACAAGGCT ATGGTTCCTA AACTAGGAAC TGGCCTTAGT TTTGACAGCA  
 TTTGTTCGTA TACCAAGGAT TTGATCCTTG ACCGGAATCA AAACGTCTGT  
 30851 CAGGTGCCAT TACAGTAGGA AACAAAAATA ATGATAAGCT AACTTTGTGG  
 GTCCACGGTA ATGTCATCCT TTGTTTTAT TACTATTGCA TTGAAACACC  
 30901 ACCACACCAG CTCCATCTCC TAACTGTAGA CTAAATGCAG AGAAAGATGC  
 TGGTGTGGTC GAGGTAGAGG ATTGACATCT GATTTACGTC TCTTTCTACG  
 30951 TAACTCACT TTGGTCTTAA CAAAATGTGG CAGTCAAATA CTTGCTACAG  
 ATTTGAGTGA AACCAGAATT GTTTTACACC GTCAGTTTAT GAACGATGTC  
 31001 TTTCAAGTTT GGCTGTAAA GGCAGTTTGG CTCCAATATC TGGAACAGTT  
 AAAGTCAAAA CCGACAATTT CCGTCAAACC GAGGTTATAG ACCTTGTCOA  
 31051 CAAAGTGCTC ATCTTATTAT AAGATTGAC GAAAATGGAG TGCTACTAAA  
 GTTTCACGAG TAGAATAATA TTCTAACTG CTTTACCTC ACGATGATTT  
 31101 CAATTCCTTC CTGGACCCAG AATATTGAA CTTTAGAAAT GGAGATCTTA  
 GTTAAGGAAG GACCTGGGTC TTATAACCTT GAAATCTTTA CCTCTAGAAT  
 31151 CTGAAGGCAC AGCCTATACA AACGCTGTTG GATTTATGCC TAACCTATCA  
 GACTTCCGTG TCGGATATGT TTGCGACAAC CTAAATACCG ATTGGATAGT  
 31201 GCTTATCCAA AATCTCACGG TAAAACCTGCC AAAAGTAACA TTGTCAGTCA  
 CGAATAGGTT TTAGAGTGCC ATTTTGACGG TTTTATTGT AACAGTCAGT

Figure 27 AG

31251 AGTTTACTTA AAGGAGACA AAACATAACC TGTAACACTA ACCATTACAC  
 TCAAATGAAT TTGCCTCTGT TTTGATTGTT ACATTGTGAT TGGTAATGTG

31301 TAAACGGTAC ACAGGAAACA GGAGACACAA CTCCAAGTGC ATACTCTATG  
 ATTTGCCATG TGTCCTTTGT CCTCTGTGTT GAGGTTACAG TATGAGATAC

31351 TCATTTTCAT GGGACTGGTC TGGCCACAAC TACATTAATG AAATATTGTC  
 AGTAAAGTA CCCTGACCAG ACCGGTGTG ATGTAATTAC TTTATAAACG

31401 CACATCCTCT TACACTTTTT CATACATTGC CCAAGAATAA AGAATCGTTT  
 GTGTAGGAGA ATGTGAAAAA GTATGTAACG GGTCTCTATT TCTTAGCAAA

31451 GTGTTATGTT TCAACGTGTT TATTTTTCAA TTGCAGAAAA TTTCAAGTCA  
 CACAATACAA AGTTGCACAA ATAAAAAGTT AACGTCTTTT AAAGTTCAGT

31501 TTTTTCATTG AGTAGTATAG CCCCACCACC ACATAGCTTA TACAGATCAC  
 AAAAAGTAAG TCATCATATC GGGGTGGTGG TGTATCGAAT ATGTCTAGTG

31551 CGTACCTTAA TCAAACACAC AGAACCCTAG TATTCAACCT GCCACCTCCC  
 GCATGGAATT AGTTTGAGTG TCTTGGGATC ATAAGTTGGA CGGTGGAGGG

31601 TCCCAACACA CAGAGTACAC AGTCCTTTCT CCCCAGCTGG CCTTAAAAAG  
 AGGGTTGTGT GTCTCATGTG TCAGGAAAGA GGGGCCGACC GGAATTTTTC

31651 CATCATATCA TGGGTAACAG ACATATTCTT AGGTGTTATA TTCCACACGG  
 GTAGTATAGT ACCCATGTGC TGTATAAGAA TCCACAATAT AAGGTGTGCC

31701 TTTCTGTGCG AGCCAAACGC TCATCAGTGA TATTAATAAA CTCCCCGGGC  
 AAAGGACAGC TCGGTTTGCG AGTAGTCACT ATAATTATTT GAGGGGCCCCG

31751 AGCTCACTTA AGTTCATGTC GCTGTCCAGC TGCTGAGCCA CAGGCTGCTG  
 TCGAGTGAAT TCAAGTACAG CGACAGGTCG ACGACTCGGT GTCCGACGAC

31801 TCCAACCTGC GGTTGCTTAA CGGGCGGCGA AGGAGAAGTC CACGCCTACA  
 AGGTTGAACG CCAACGAATT GCCCGCCGCT TCCTCTTCAG GTGCGGATGT

31851 TGGGGGTAGA GTCATAATCG TGCATCAGGA TAGGGCGGTG GTGCTGCAGC  
 ACCCCCATCT CAGTATTAGC ACGTAGTCCT ATCCCGCCAC CACGACGTCG

31901 AGCGCGCGAA TAAACTGCTG CCGCCGCCGC TCCGTCTGTC AGGAATACAA  
 TCGCGCGCTT ATTTGACGAC GCGCGCGGCG AGGCAGGACG TCCTTATGTT

31951 CATGGCAGTG GTCTCCTCAG CGATGATTG CACCGCCCGC AGCATAAGGC  
 GTACCGTCAC CAGAGGAGTC GCTACTAAGC GTGGCGGGCG TCGTATTCCG

32001 GCCTTGTCTT CCGGGCACAG CAGCGCACCC TGATCTCACT TAAATCAGCA  
 CGGAACAGGA GGCCCGTGTG GTGCGGTGGG ACTAGAGTGA ATTTAGTCGT

32051 CAGTAACTGC AGCACAGCAC CACAATAATG TTCAAAATCC CACAGTGCAA  
 GTCATPGACG TCGTGTCTGT GTGTTATAAC AAGTTTTAGG GTGTCACGTT

32101 GCGCTGTAT CCAAAGCTCA TGGCGGGGAC CACAGAACCC ACGTGGCCAT  
 CCGCGACATA GGTTCGAGT ACCGCCCTG GTGTCTTGGG TGCACCGGTA

32151 CATACCACAA GCGCAGGTAG ATTAAGTGGC GACCCCTCAT AAACACGCTG  
 GTATGGTGTT CGCGTCCATC TAATTCACCG CTGGGGAGTA TTTGTGCGAC

Figure 27AH



32201 GACATAAACA TCTCTTT TGGCATGTTG TAATTCACCA CCTCCC  
 CTGTATTGT AATGGAGAAA ACCGTACAAC ATTAAGTGGT GGAGGGCCAT  
 32251 CCATATAAAC CTCTGATTAA ACATGGCGCC ATCCACCACC ATCCTAAACC  
 GGTATATTGT GAGACTAATT TGTACCGCGG TAGGTGGTGG TAGGATTGG  
 32301 AGCTGGCCAA AACCTGCCCG CCGGTATAC ACTGCAGGGA ACCGGGACTG  
 TCGACCGGTT TTGGACGGGC GGCCGATATG TGACGTCCCT TGGCCCTGAC  
 32351 GAACAATGAC AGTGGAGAGC CCAGGACTCG TAACCATGGA TCATCATGCT  
 CTTGTTACTG TCACCTCTCG GGTCTGAGC ATTGGTACCT AGTAGTACGA  
 32401 CGTCATGATA TCAATGTTGG CACAACACAG GCACACGTGC ATACACTTCC  
 GCAGTACTAT AGTTACAACC GTGTTGTGTC CGTGTGCACG TATGTGAAGG  
 32451 TCAGGATTAC AAGCTCCTCC CGCGTTAGAA CCATATCCCA GGAACAACC  
 AGTCCTAATG TTCGAGGAGG GCGCAATCTT GGTATAGGGT CCCTTGTGG  
 32501 CATTCCTGAA TCAGCGTAAA TCCCACACTG CAGGGAAGAC CTCGCACGTA  
 GTAAGGACTT AGTCGCATTT AGGGTGTGAC GTCCCTTCTG GAGCGTGCAT  
 32551 ACTCACGTTG TGCATTGTCA AAGTGTTACA TTCGGGCAGC AGCGGATGAT  
 TGAGTGCAAC ACGTAACAGT TTCACAATGT AAGCCCGTCG TCGCCTACTA  
 32601 CCTCCAGTAT GGTAGCGCGG GTTCTGTCT CAAAAGGAGG TAGACGATCC  
 GGAGGTCATA CCATCGCGCC CAAAGACAGA GTTTCTCTCC ATCTGCTAGG  
 32651 CTACTGTACG GAGTGCGCCG AGACAACCGA GATCGTGTG STCGTAGTGT  
 GATGACATGC CTCACGCGGC TCTGTTGSC TTAGCACAAC CAGCATCACA  
 32701 CATGCCAAAT GGAACGCCGG ACGTAGTCAT ATTTCTGAA GCAAAACCAG  
 GTACGGTTTA CCTTGCGGCC TGCATCAGTA TAAAGGACTT CGTTTTGGTC  
 32751 GTGCGGGCGT GACAAACAGA TCTGCGTCTC CGGTCTCGCC GCTTAGATCG  
 CACGCCCCGA CTGTTTGTCT AGACGCAGAG GCCAGAGCGG CGAATCTAGC  
 32801 CTCTGTGTAG TAGTTGTAGT ATATCCAATC TCTCAAAGCA TCCAGGCGCC  
 GAGACACATC ATCAACATCA TATAGGTGAG AGAGTTTCGT AGGTCCGCGG  
 32851 CCCTGGCTTC GGGTTCATG TAAACTCCTT CATGCGCCGC TGCCCTGATA  
 GGGACCGAAG CCCAAGATAC ATTTAGGAA GTACGCGCGC ACGGGACTAT  
 32901 ACATCCACCA CCGCAGAATA AGCCACACCC AGCCAACCTA CACATTGCTT  
 TGTAGGTGGT GGCCTCTTAT TCGGTGTGGG TCGGTGGAT GTGTAAGCAA  
 32951 CTGCGAGTCA CACACGGGAG GAGCGGGAAG AGCTGGAAGA ACCATGTTTT  
 GACGCTCAGT GTGTGCCCTC CTCGCCCTTC TCGACCTTCT TGGTACAAAA  
 33001 TTTTTTTATT CCAAAAGATT ATCCAAAACC TCAAAATGAA GATCTATTAA  
 AAAAAATAA GGTTTTCTAA TAGGTTTTGG AGTTTTACTT CTAGATAATT  
 33051 GTGAACGCGC TCCCCTCCGG TGGCGTGGTC AAATCTTACA GCCAAAGAAC  
 CACTTGCGCG AGGGGAGGCC ACCGCACCAG TTTGAGATGT CGGTTTCTTG  
 33101 AGATAATGGC ATTTGTAAGA TGTGCACAA TGGCTTCAA AAGGCAACG  
 TCTATTACCG TAAACATTCT ACAACGTGTT ACCGAAGGTT TTCCGTTTGC

Figure 27 AI

33151 GCCCTCACGT GTGGAC GTAAAGGCTA AACCTTCAG GGTGAATTC  
 CGGGAGTGCA GTTCACCTG CATTTCCGAT TTGGGAAGTC CCACTTTAG  
 33201 CTCTATAAAC ATTCCAGCAC CTTCAACCAT GCCCAAATAA TTCTCATCTC  
 GAGATATTTG TAAGGTCGTG GAAGTTGGTA CGGGTTTATT AAGAGTAGAG  
 33251 GCCACCTTCT CAATATATCT CTAAGCAAAT CCCGAATATT AAGTCCGGCC  
 CGGTGGAAGA GTTATATAGA GATTCGTTTA GGGCTTATAA TTCAGGCCGG  
 33301 ATTGTAAAAA TCTGCTCCAG AGCGCCCTCC ACCTTCAGCC TCAAGCAGCG  
 TAACATTTTT AGACGAGGTC TCGCGGGAGG TGGGAAGTCGG AGTTCGTCGC  
 33351 AATCATGATT GCAAAAATTC AGGTTCCTCA CAGACCTGTA TAAGATTCAA  
 TTAGTACTAA CGTTTTTAAG TCCAAGGAGT GTCTGGACAT ATTCTAAGTT  
 33401 AAGCGGAACA TTAACAAAAA TACCGCGATC CCGTAGGTCC CTTGCGAGGG  
 TTCGCTTGT AATTGTTTTT ATGGCGCTAG GGCATCCAGG GAAGCGTCCC  
 33451 CCAGCTGAAC ATAATCGTGC AGGTCTGCAC GGACCAGCGC GGCCACTTCC  
 GGTGCGACTG TATTAGCACG TCCAGACGTG CCTGGTCGCG CCGGTGAAGG  
 33501 CCGCCAGGAA CCATGACAAA AGAACCACCA CTGATTATGA CACGCATACT  
 GCGGCTCCTT GGTACTGTTT TCTTGGGTGT GACTAATACT GTGCGTATGA  
 33551 CGGAGCTATG CTAACCAGCG TAGCCCCGAT GTAAGCTTGT TGCATGGGCG  
 GCCTCGATAC GATTGCTCGC ATCGGGGCTA CATTCGAACA ACGTACCCGC  
 33601 GCGATATAAA ATGCAAGGTG CTGCTCAAAA AATCAGGCAA AGCCTCGCGC  
 CGCTATATTT TACGTTCCAC GACGAGTTTT TTAGTCCGTT TCGGAGCGCG  
 33651 AAAAAAGAAA GCACATCGTA GTCATGCTCA TGCAGATAAA GGCAGGTAAG  
 TTTTTCTTT CGTGTAGCAT CAGTACGAGT ACGTCTATTT CCGTCCATTC  
 33701 CTCCGGAACC ACCACAGAAA AAGACACCAT TTTTCTCTCA AACATGTCTG  
 GAGGCCTTGG TGGTGTCTTT TTCTGTGCTA AAAAGAGAGT TTGTACAGAC  
 33751 CGGGTTTCTG CATAAACACA AAATAAAATA ACAAAAAAAC ATTTAAACAT  
 GCCCAAAGAC GTATTTGTGT TTTATTTTAT TGTTTTTTTG TAAATTTGTA  
 33801 TAGAAGCCTG TCTTACAACA GGAAAAACAA CCCTTATAAG CATAAGACGG  
 ATCTTCGGAC AGAATGTTGT CCTTTTGTGTT GGAATATTC GTATTCTGCC  
 33851 ACTACGGCCA TGCCGGCGTG ACCGTAAAAA AACTGGTCAC CGTGATTAAA  
 TGATGCCGGT ACGGCCGCAC TGGCATTTTT TTGACCAGTG GCACTAATTT  
 33901 AAGCACCACC GACAGCTCCT CGGTCAATGC CGGAGTCATA ATGTAAGACT  
 TTCGTGGTGG CTGTCGAGGA GCCAGTACAG GCCTCAGTAT TACATTCTGA  
 33951 CGGTAAACAC ATCAGGTTGA TTCACATCGG TCAGTGCTAA AAAGCGACCG  
 GCCATTGTG TAGTCCAAC T AAGTGTAGCC AGTCACGATT TTTGCTGGC  
 34001 AAATAGCCCG GGGGAATACA TACCCGCAGG CGTAGAGACA ACATTACAGC  
 TTTATCGGGC CCCCTTATGT ATGGGCGTCC GCATCTCTGT TGTAATGTCG  
 34051 CCCCATAGGA GGTATAACAA AATTAATAGG AGAGAAAAAC ACATAACAC  
 GGGGTATCCT CCATATTGTT TTAATTATCC TCTCTTTTTG TGTATTTGTG

Figure 27A J

34101 CTGAAAAACC CTTTGCCTA GGC AAAATAG CACCCCTCCG TCTCAGTAA  
 GACTTTTTTG GACGGAT CCGTTTATC GTGGGAGGGC GAGGTCCT  
 34151 ACATACAGCG CTTCCACAGC GGCAGCCATA ACAGTCAGCC TTACCAGTAA  
 TGTATGTCGC GAAGGTGTCG CCGTCGGTAT TGTGAGTCGG AATGGTCATT  
 34201 AAAAGAAAAC CTATTAAAA AACACCACTC GACACGGCAC CAGCTCAATC  
 TTTTCTTTTG GATAATTTT TTGTGGTGAG CTGTGCCGTG GTCGAGTTAG  
 34251 AGTCACAGTG TAAAAAGGG CCAAGTGCAG AGCGAGTATA TATAGGACTA  
 TCAGTGTCAC ATTTTTCCTC GGTTCACGTC TCGCTCATAT ATATCCTGAT  
 34301 AAAATGACG TAACGGTTAA AGTCCACAAA AAACACCCAG AAAACCGCAC  
 TTTTACTGC ATTGCCAATT TCAGGTGTTT TTGTGGGTC TTTGGCGTG  
 34351 GCGAACCTAC GCCCAGAAAC GAAAGCCAAA AAACCCACAA CTTCCTCAAA  
 CGCTTGGATG CGGGTCTTTG CTTTCGGTTT TTTGGGTGTT GAAGGAGTTT  
 34401 TCGTCACTTC CGTTTTCCTA CGTTACGTCA CTTCCTATTT TAAGAAACT  
 AGCAGTGAAG GCAAAGGGT GCAATGCAGT GAAGGGTAAA ATTCTTTTGA  
 34451 ACAATTCCCA ACACATACAA GTTACTCCGC CCTAAACCT ACCTCAGCCG  
 TGTAAGGGT TGTGTATGTT CAATGAGGCG GGATTTTGA TGCAGTGGGC  
 34501 CCCCCTTCCC ACGCCCCGCG CCACGTCACA AACTCCACCC CCTCATTATC  
 GGGCAAGGG TCGGGGGCGC GGTGCAGTGT TGAAGGTGGG GGAGTAATAG

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34551 ATATTGGCTT CAATCCAAAA TAAGGTATAT TATTGATGAT GTTAATTAAG  
 TATAACCGAA GTTAGGTTTT ATTCCATATA ATAACCTACTA CAATTAATTC  
 34601 AATTGCGATC TCGCAGCGCA GGCTGGATGG CCTTCCCAT TATGATTCTT  
 TTAAGCCTAG ACGCTGCGCT CCGACCTACC GGAAGGGGTA ATACTAAGAA  
 34651 CTCGCTTCCG GCGGCATCGG GATGCCCGCG TTGCAGGCCA TGCTGTCCAG  
 GAGCGAAGGC CGCCGTAGCC CTACGGGCGC AACGTCCGGT ACGACAGGTC  
 34701 GCAGGTAGAT GACGACCATC AGGGACAGCT TCAAGGCCAG CAAAAGGCCA  
 CGTCCATCTA CTGCTGGTAG TCCCTGTGCA AGTTCCGGTC GTTTTCCGGT  
 34751 GGAACCGTAA AAAGGCCGCG TTGCTGGCGT TTTTCCATAG GCTCCGCCCC  
 CTTTGGCATT TTTCCGGCGC AACGACCGCA AAAAGGTATC CGAGGCGGGG  
 34801 CCTGACGAGC ATCACAAAAA TCGACGCTCA AGTCAGAGGT GCGGAAACCC  
 GGACTGCTCG TAGTGTTTT AGCTGCGAGT TCAGTCTCCA CCGCTTTGGG  
 34851 GACAGGACTA TAAAGATACC AGGCGTTTCC CCCTGGAAGC TCCCTCGTGC  
 CTGTCTGAT ATTTCTATGG TCCGCAAGG GGGACCTTCG AGGGAGCACG  
 34901 GCTCTCTGT TCCGACCCTG CCGCTTACCG GATACCTGTC CGCCTTTCTC  
 CGAGAGGACA AGGCTGGGAC GCGCAATGGC CTATGGACAG GCGGAAAGAG  
 34951 CCTTCGGGAA GCGTGCGCT TTCTCATAGC TCACGCTGTA GGTATCTCAG  
 GGAAGCCCTT CGCACCAGCA AAGAGTATCG AGTGCGACAT CCATAGAGTC  
 35001 TTCGGGTAG GTCGTTGCT CCAAGCTGGG CTGTGTGCAC GAACCCCCCG  
 AAGCCACATC CAGCAAGCGA GGTTCGACCC GACACACGTG CTTGGGGGGC

Figure 27 AK

35051 TTCAGCCCGA CCGCTGCGCC TTATCCGGTA ACTATCGTCT TGAGTCCAAC  
 AAGTCGGGCT GGCACGCGG AATAGGCCAT TGATAGCAGA ACTCAGGTTG

35101 CCGGTAAGAC ACGACTTATC GCCACTGGCA GCAGCCACTG GTAACAGGAT  
 GGCCATTCTG TGCTGAATAG CCGTGACCGT CGTCGGTGAC CATTGTCCTA

35151 TAGCAGAGCG AGGTATGTAG GCGGTGCTAC AGAGTTCTTG AAGTGGTGGC  
 ATCGTCTCGC TCCATACATC CGCCACGATG TCTCAAGAAC TTCACCACCG

35201 CTAACCTACGG CTACACTAGA AGGACAGTAT TTGGTATCTG CGCTCTGCTG  
 GATTGATGCC GATGTGATCT TCCTGTCATA AACCATAGAC GCGAGACGAC

35251 AAGCCAGTTA CCTTCGGAAA AAGAGTTGGT AGCTCTTGAT CCGGCAAACA  
 TTCGGTCAAT GGAAGCCTTT TTCTCAACCA TCGAGAACTA GGCCGTTTGT

35301 AACCACCGCT GGTAGCGGTG GTTTTTTTGT TTGCAAGCAG CAGATTACGC  
 TTGGTGGCGA CCATCGCCAC CAAAAAACA AACGTTCTGTC GTCTAATGCG

35351 GCAGAAAAAA AGGATCTCAA GAAGATCCTT TGATCTTTTC TACGGGGTCT  
 CGTCTTTTTT TCCTAGAGTT CTTCTAGGAA ACTAGAAAAG ATGCCCCAGA

35401 GACGCTCAGT GGAACGAAAA CTCACGTTAA GGGATTTTGG TCATGAGATT  
 CTGCGAGTCA CCTTGCTTTT GAGTGCAATT CCCTAAACC AGTACTCTAA

35451 ATCAAAAAGG ATCTTCACCT AGATCCTTTT AAATCAATCT AAAGTATATA  
 TAGTTTTTCC TAGAAGTGGA TCTAGGAAAA TTTAGTTAGA TTTCATATAT

35501 TGAGTAACT TGGTCTGACA GTTACCAATG CTTAATCAGT GAGGCACCTA  
 ACTCATTTGA ACCAGACTGT CAATGGTTAC GAATTAGTCA CTCCGTGGAT

35551 TCTCAGCGAT CTGTCTATTT CGTTCATCCA TAGTTGCCCTG ACTCCCCGTC  
 AGAGTCGCTA GACAGATAAA GCAAGTAGGT ATCAACGGAC TGAGGGGCAG

35601 GTGTAGATAA CTACGATACG GGAGGGCTTA CCATCTGGCC CCAGTGCTGC  
 CACATCTATT GATGCTATGC CCTCCGAAT GGTAGACCGG GGTCACGACG

35651 AATGATACCG CGAGACCCAC GCTCACCAGC TCCAGATTTA TCAGCAATAA  
 TTACTATGGC GCTCTGGGTG CGAGTGGCCG AGGTCTAAAT AGTCGTTATT

35701 ACCAGCCAGC CGGAAGGGCC GAGCGCAGAA GTGGTCCTGC AACTTTATCC  
 TGGTCGGTCG GCCTTCCCGG CTCGCGTCTT CACCAGGACG TTGAAATAGG

35751 GCCTCCATCC AGTCTATTAA TTGTTGCCGG GAAGCTAGAG TAAGTAGTTC  
 CGGAGGTAGG TCAGATAATT AACAACGGCC CTTGATCTC ATTCATCAAG

35801 GCCAGTTAAT AGTTTGCACA ACGTTGTTGC CATTGCTACA GGCACTGTGG  
 CGGTCAATTA TCAAACGCGT TGCAACAACG GTAACGATGT CCGTAGCACC

35851 TGTCACGCTC GTCGTTTGGT ATGGCTTCAT TCAGCTCCGG TTCCCAACGA  
 ACAGTGCGAG CAGCAAACCA TACCGAAGTA AGTCGAGGCC AAGGGTTGCT

35901 TCAAGGCGAG TTACATGATC CCCCATGTTG TGCAAAAAAG CGGTAGCTC  
 AGTTCCGCTC AATGTACTAG GGGGTACAAC ACGTTTTTTC GCCAATCGAG

35951 CTTCCGGTCTT CCGATCGTTG TCAGAAGTAA GTTGGCCGCA GTGTTATCAC  
 GAAGCCAGGA GGCTAGCAAC AGTCTTCATT CAACCGGCGT CACAATAGTG

Figure 2 AL

36001 TCATGGTTAT ~~GG~~AGCACTG CATAATTCTC TTACTGTCAT GCCATC~~TA~~  
AGTACCAATA CCGTCGTGAC GTATTAAGAG AATGACAGTA CGGTAGGCAT

36051 AGATGCTTTT CTGTGACTGG TGAGTACTCA ACCAAGTCAT TCTGAGAATA  
TCTACGAAAA GACACTGACC ACTCATGAGT TGGTTCAGTA AGACTCTTAT

36101 GTGTATGCGG CGACCGAGTT GCTCTTGCCC GCGTCAACA CGGGATAATA  
CACATACGCC GCTGGCTCAA CGAGAACGGG CCGCAGTTGT GCCCTATTAT

36151 CCGCGCCACA TAGCAGAACT TTAAGAGTGC TCATCATTGG AAAACGTTCT  
GGCGCGGTGT ATCGTCTTGA AATTTTCACG AGTAGTAACC TTTTGCAAGA

36201 TCGGGGCGAA AACTCTCAAG GATCTTACCG CTGTTGAGAT CCAGTTCGAT  
AGCCCCGCTT TTGAGAGTTC CTAGAATGGC GACAACTCTA GGTCAAGCTA

36251 GTAACCCACT CGTGCACCCA ACTGATCTTC AGCATCTTTT ACTTTCACCA  
CATTGGGTGA GCACGTGGGT TGA TAGAAG TCGTAGAAAA TGAAAGTGGT

36301 GCGTTTCTGG GTGAGCAAAA ACAGGAAGGC AAAATGCCGC AAAAAAGGGA  
CGCAAAGACC CACTCGTTTT TGTCTTCCG TTTTACGGCG TTTTTCCT

36351 ATAAGGGCGA CACGGAAATG TTGAATACTC ATACTCTTCC TTTTCAATA  
TATTCCTGCT GTGCCTTTAC AACTTATGAG TATGAGAAGG AAAAAGTTAT

36401 TTATTGAAGC ATTTATCAGG GTTATTGTCT CATGAGCGGA TACATATTTG  
AATAACTTCG TAAATAGTCC CAATAACAGA GTACTCGCCT ATGTATAAAC

36451 AATGTATTTA GAAAAATAAA CAAATAGGGG TTCCGCGCAC ATTTCCCCGA  
TTACATAAAT CTTTTTATTT GTTTATCCCC AAGGCGCGTG TAAAGGGGCT

36501 AAAGTGCCAC CTGACGTCTA AGAAACCATT ATTATCATGA CATTACCTA  
TTTCACGGTG GACTGCAGAT TCTTTGGTAA TAATAGTACT GTAATTGGAT

36551 TAAAAATAGG CGTATCACGA GGCCCTTTCG TCTTCAAGAA TTGGATCCGA  
ATTTTATCC GCATAGTGCT CCGGGAAAGC AGAAGTTCTT AACCTAGGCT

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36601 ATTCTTAATT TCTTAATTAA (SEQ ID NO:34)  
TAAGAATTAA AGAATTAATT (SEQ ID NO:35)

*Figure 27A M*

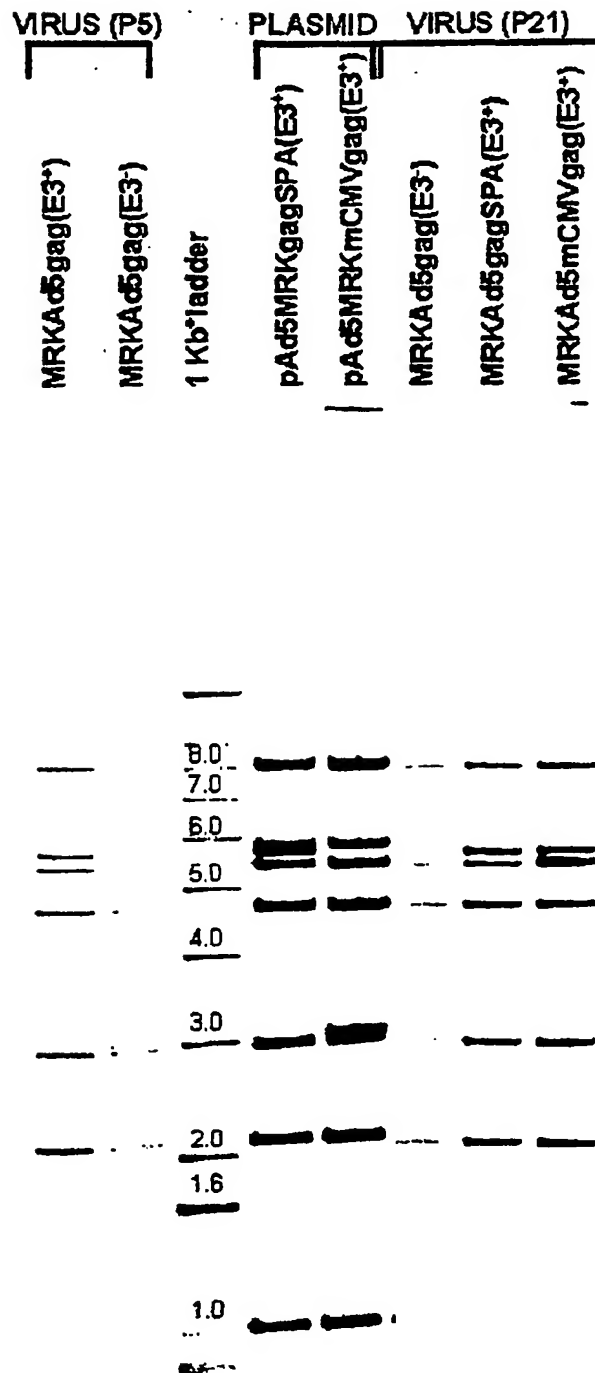


FIGURE 28

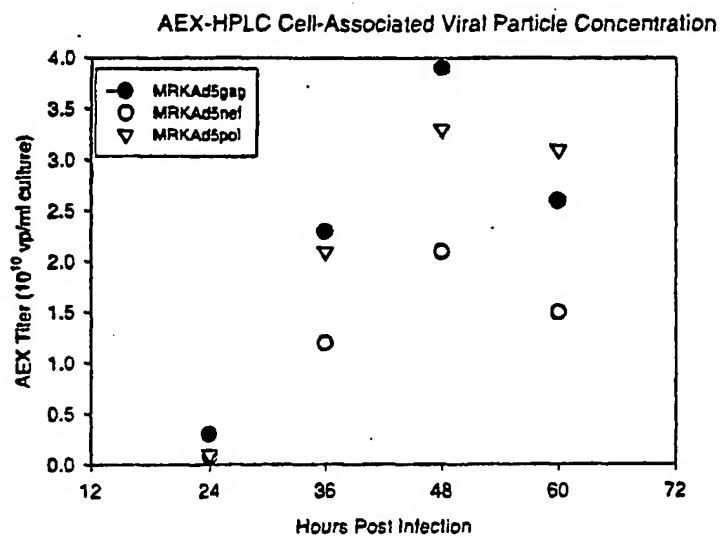


FIGURE 29A

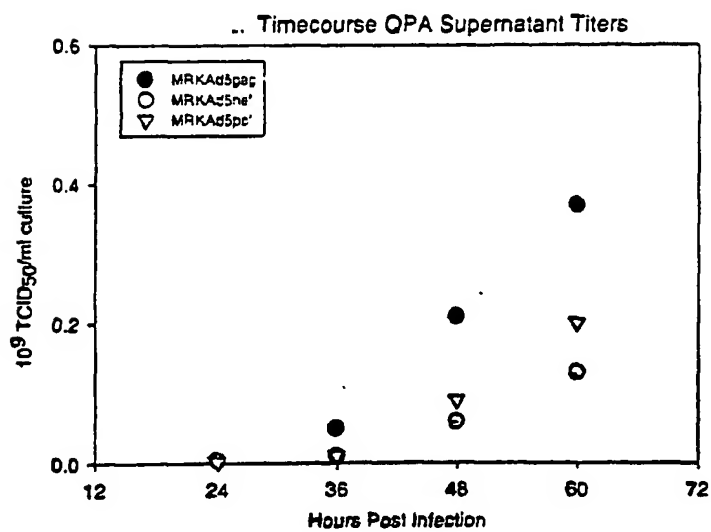


FIGURE 29B

atg gat gca atg aag aga ggg ctc tgc tgt gtg ctg ctg ctg tgt gga Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly 1 5 10 15	48
gca gtc ttc gtt tct ccc agc gag atc tcc att gtg tgg gcc tcc agg Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ile Val Trp Ala Ser Arg 20 25 30	96
gag ctg gag agg ttt gct gtg aac cct ggc ctg ctg gag acc tct gag Glu Leu Glu Arg Phe Ala Val Asn Pro Gly Leu Leu Glu Thr Ser Glu 35 40 45	144
ggg tgc agg cag atc ctg ggc cag ctc cag ccc tcc ctg caa aca ggc Gly Cys Arg Gln Ile Leu Gly Gln Leu Gln Pro Ser Leu Gln Thr Gly 50 55 60	192
tct gag gag ctg agg tcc ctg tac aac aca gtg gct acc ctg tac tgt Ser Glu Glu Leu Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys 65 70 75 80	240
gtg cac cag aag att gat gtg aag gac acc aag gag gcc ctg gag aag Val His Gln Lys Ile Asp Val Lys Asp Thr Lys Glu Ala Leu Glu Lys 85 90 95	288
att gag gag gag cag aac aag tcc aag aag aag gcc cag cag gct gct Ile Glu Glu Glu Gln Asn Lys Ser Lys Lys Lys Ala Gln Gln Ala Ala 100 105 110	336
gct ggc aca ggc aac tcc agc cag gtg tcc cag aac tac ccc att gtg Ala Gly Thr Gly Asn Ser Ser Gln Val Ser Gln Asn Tyr Pro Ile Val 115 120 125	384
cag aac ctc cag ggc cag atg gtg cac cag gcc atc tcc ccc cgg acc Gln Asn Leu Gln Gly Gln Met Val His Gln Ala Ile Ser Pro Arg Thr 130 135 140	432
ctg aat gcc tgg gtg aag gtg gtg gag gag aag gcc ttc tcc cct gag Leu Asn Ala Trp Val Lys Val Val Glu Glu Lys Ala Phe Ser Pro Glu 145 150 155 160	480
gtg atc ccc atg ttc tct gcc ctg tct gag ggt gcc acc ccc cag gac Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala Thr Pro Gln Asp 165 170 175	528
ctg aac acc atg ctg aac aca gtg ggg ggc cat cag gct gcc atg cag Leu Asn Thr Met Leu Asn Thr Val Gly Gly His Gln Ala Ala Met Gln 180 185 190	576
atg ctg aag gag acc atc aat gag gag gct gct gag tgg gac agg ctg Met Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala Glu Trp Asp Arg Leu 195 200 205	624
cat cct gtg cac gct ggc ccc att gcc ccc ggc cag atg agg gag ccc His Pro Val His Ala Gly Pro Ile Ala Pro Gly Gln Met Arg Glu Pro 210 215 220	672
agg ggc tct gac att gct ggc acc acc tcc acc ctc cag gag cag att Arg Gly Ser Asp Ile Ala Gly Thr Thr Ser Thr Leu Gln Glu Gln Ile 225 230 235 240	720
ggc tgg atg acc aac aac ccc ccc atc cct gtg ggg gaa atc tac aag Gly Trp Met Thr Asn Asn Pro Pro Ile Pro Val Gly Glu Ile Tyr Lys 245 250 255	768

Figure 30'A



agg tgg atc atc ctg ggc ctg aac aag att gtg agg atg tac tcc ccc	816
Arg Trp Ile Ile Leu Gly Leu Asn Lys Ile Val Arg Met Tyr Ser Pro	
260 265 270	
acc tcc atc ctg gac atc agg cag ggc ccc aag gag ccc ttc agg gac	864
Thr Ser Ile Leu Asp Ile Arg Gln Gly Pro Lys Glu Pro Phe Arg Asp	
275 280 285	
tat gtg gac agg ttc tac aag acc ctg agg gct gag cag gcc tcc cag	912
Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala Glu Gln Ala Ser Gln	
290 295 300	
gag gtg aag aac tgg atg aca gag acc ctg ctg gtg cag aat gcc aac	960
Glu Val Lys Asn Trp Met Thr Glu Thr Leu Leu Val Gln Asn Ala Asn	
305 310 315 320	
cct gac tgc aag acc atc ctg aag gcc ctg ggc cct gct gcc acc ctg	1008
Pro Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro Ala Ala Thr Leu	
325 330 335	
gag gag atg atg aca gcc tgc cag ggg gtg ggg ggc cct ggt cac aag	1056
Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly Pro Gly His Lys	
340 345 350	
gcc agg gtg ctg gct gag gcc atg tcc cag gtg acc aac tcc gcc acc	1104
Ala Arg Val Leu Ala Glu Ala Met Ser Gln Val Thr Asn Ser Ala Thr	
355 360 365	
atc atg atg cag agg ggc aac ttc agg aac cag agg aag aca gtg aag	1152
Ile Met Met Gln Arg Gly Asn Phe Arg Asn Gln Arg Lys Thr Val Lys	
370 375 380	
tgc ttc aac tgt ggc aag gtg ggc cac att gcc aag aac tgt agg gcc	1200
Cys Phe Asn Cys Gly Lys Val Gly His Ile Ala Lys Asn Cys Arg Ala	
385 390 395 400	
ccc agg aag aag ggc tgc tgg aag tgt ggc aag gag ggc cac cag atg	1248
Pro Arg Lys Lys Gly Cys Trp Lys Cys Gly Lys Glu Gly His Gln Met	
405 410 415	
aag gac tgc aat gag agg cag gcc aac ttc ctg ggc aaa atc tgg ccc	1296
Lys Asp Cys Asn Glu Arg Gln Ala Asn Phe Leu Gly Lys Ile Trp Pro	
420 425 430	
tcc cac aag ggc agg cct ggc aac ttc ctc cag tcc agg cct gag ccc	1344
Ser His Lys Gly Arg Pro Gly Asn Phe Leu Gln Ser Arg Pro Glu Pro	
435 440 445	
aca gcc cct ccc gag gag tcc ttc agg ttt ggg gag gag aag acc acc	1392
Thr Ala Pro Pro Glu Glu Ser Phe Arg Phe Gly Glu Glu Lys Thr Thr	
450 455 460	
ccc agc cag aag cag gag ccc att gac aag gag ctg tac ccc ctg gcc	1440
Pro Ser Gln Lys Gln Glu Pro Ile Asp Lys Glu Leu Tyr Pro Leu Ala	
465 470 475 480	
tcc ctg agg tcc ctg ttt ggc aac gac ccc tcc tcc cag taa (SID NO:36)	1482
Ser Leu Arg Ser Leu Phe Gly Asn Asp Pro Ser Ser Gln * (SID NO:37)	
485 490	

Figure 30 B

**Figure 31**

**IFN- $\gamma$  Secretion against Gag 20-aa pool from CD3<sup>+</sup> T cells of Monkey PBMCs**

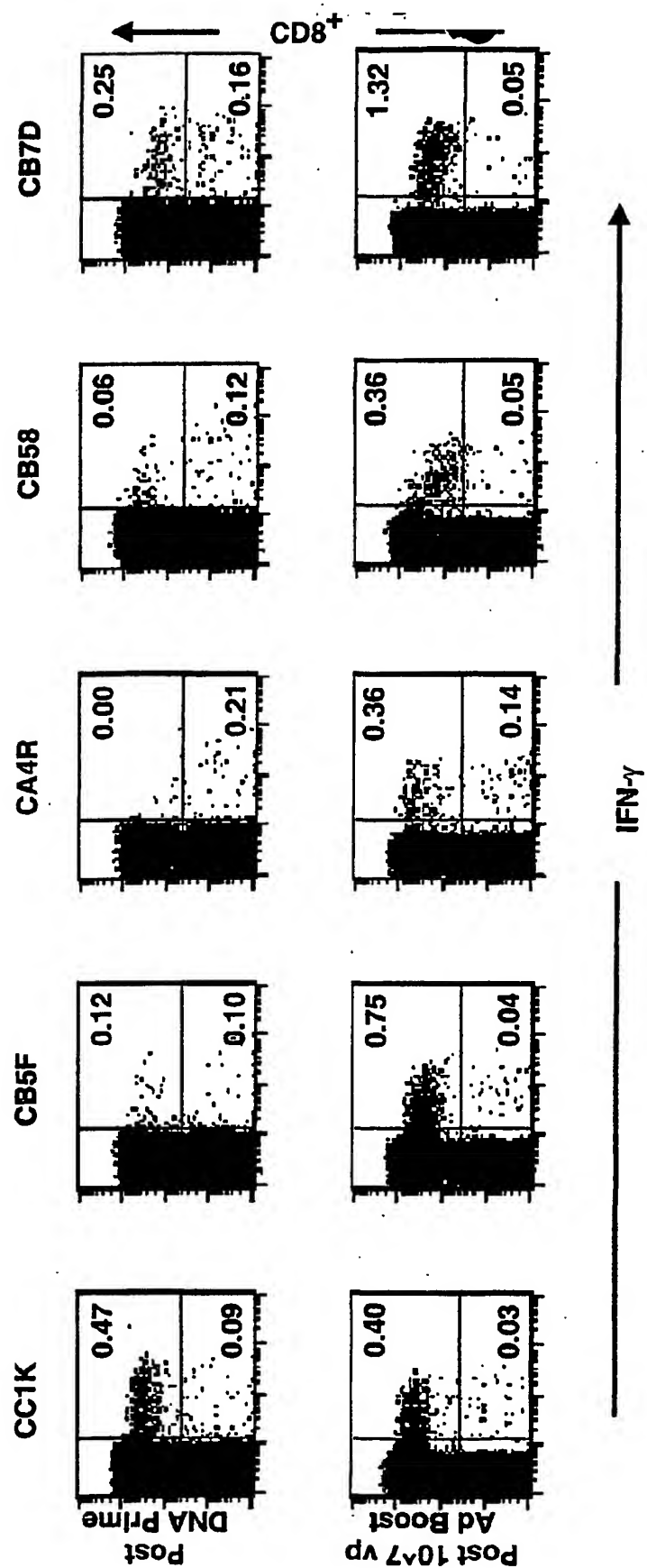


FIGURE 32

# Comparison of Single-Modality Adenovirus Immunization with DNA+Adjuvant Prime/Adenovirus Boost

## Immunizations

Ad Prime/Boost

DNA-CRL1005 Prime/Ad Boost

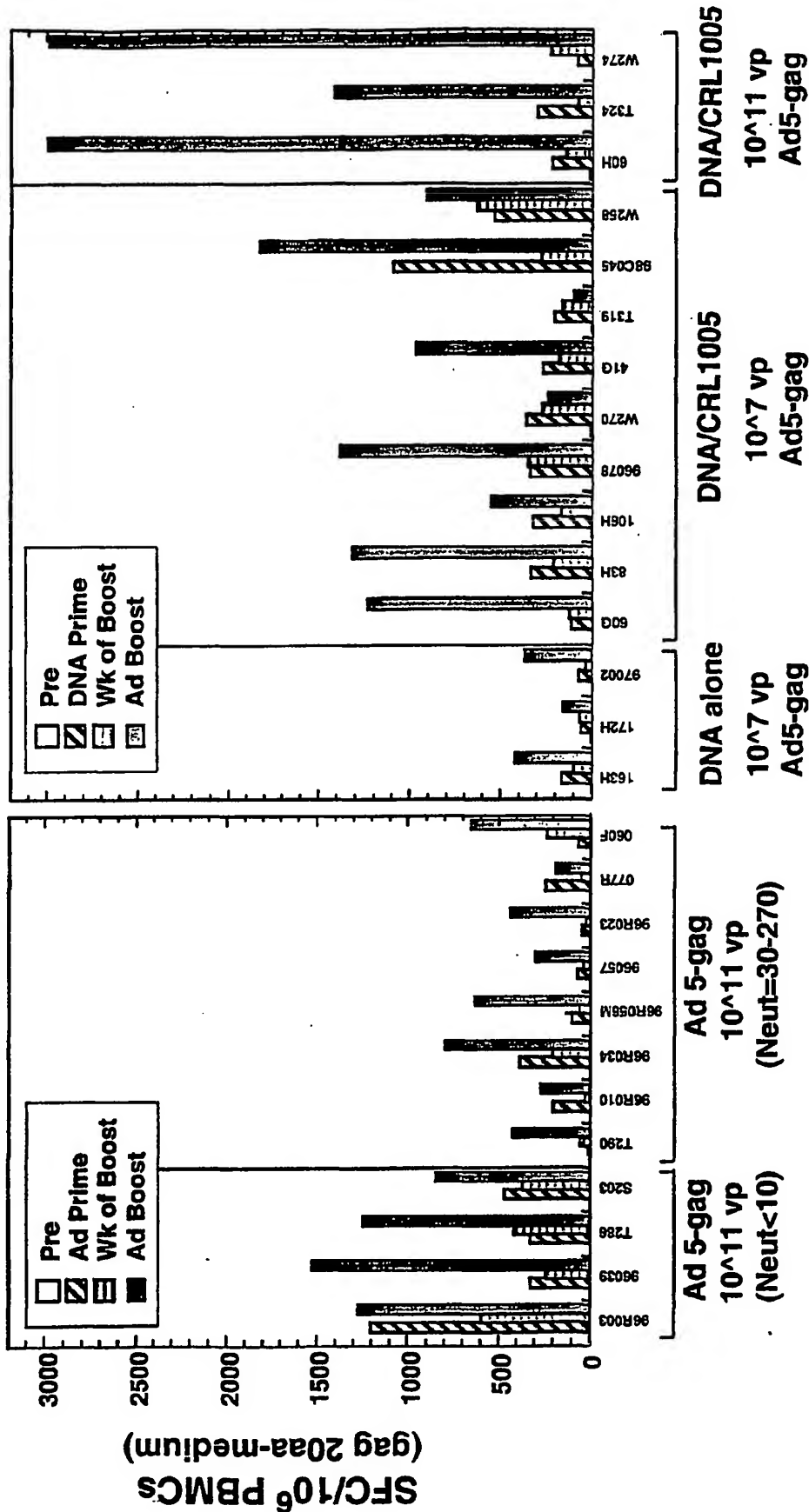


FIGURE 33A

ATGGGTGCTA GGGCTTCTGT GCTGTCTGGT GGTGAGCTGG ACAAGTGGGA GAAGATCAGG  
 CTGAGGCCTG GTGGCAAGAA GAAGTACAAG CTAAAGCACA TTGTGTGGGC CTCCAGGGAG  
 CTGGAGAGGT TTGCTGTGAA CCCTGGCCTG CTGGAGACCT CTGAGGGGTG CAGGCAGATC  
 CTGGGCCAGC TCCAGCCCTC CCTGCAAACA GGCTCTGAGG AGCTGAGGTC CCTGTACAAC  
 ACAGTGGCTA CCCTGTACTG TGTGCACCAG AAGATTGATG TGAAGGACAC CAAGGAGGCC  
 CTGGAGAAGA TTGAGGAGGA GCAGAACAAG TCCAAGAAGA AGGCCAGCA GGCTGCTGCT  
 GGCACAGGCA ACTCCAGCCA GGTGTCCCAG AACTACCCCA TTGTGCAGAA CCTCCAGGGC  
 CAGATGGTGC ACCAGGCCAT CTCCCCCGG ACCCTGAATG CCTGGGTGAA GGTGGTGGAG  
 GAGAAGGCCT TCTCCCTGA GGTGATCCCC ATGTTCTCTG CCCTGTCTGA GGGTGCCACC  
 CCCCAGGACC TGAACACCAT GCTGAACACA GTGGGGGGCC ATCAGGCTGC CATGCAGATG  
 CTGAAGGAGA CCATCAATGA GGAGGCTGCT GAGTGGGACA GGCTGCATCC TGTGCACGCT  
 GGCCCCATTG CCCCCGGCCA GATGAGGGAG CCCAGGGGCT CTGACATTGC TGGCACCACC  
 TCCACCCTCC AGGAGCAGAT TGGCTGGATG ACCAACAACC CCCCATCCC TGTGGGGGAA  
 ATCTACAAGA GGTGGATCAT CCTGGGCCTG AACAAGATTG TGAGGATGTA CTCCCCCACC  
 TCCATCCTGG ACATCAGGCA GGGCCCCAAG GAGCCCTTCA GGGACTATGT GGACAGGTTC  
 TACAAGACCC TGAGGGCTGA GCAGGCCTCC CAGGAGGTGA AGAACTGGAT GACAGAGACC  
 CTGCTGGTGC AGAATGCCAA CCCTGACTGC AAGACCATCC TGAAGGCCCT GGGCCCTGCT  
 GCCACCCTGG AGGAGATGAT GACAGCCTGC CAGGGGGTGG GGGGCCCTGG TCACAAGGCC  
 AGGGTGCTGG CTGAGGCCAT GTCCCAGGTG ACCAACTCCG CCACCATCAT GATGCAGAGG  
 GGCAACTTCA GGAACCAGAG GAAGACAGTG AAGTGCTTCA ACTGTGGCAA GGTGGGCCAC  
 ATTGCCAAGA ACTGTAGGGC CCCCAGGAAG AAGGGCTGCT GGAAGTGTGG CAAGGAGGGC  
 CACCAGATGA AGGACTGCAA TGAGAGGCAG GCCTAACTTCC TGGGCAAAAT CTGGCCCTCC  
 CACAAGGGCA GGCTTGCAA CTTCTCCAG TCCAGGCCTG AGCCCACAGC CCTTCCCGAG  
 GAGTCCTTCA GGTTTGGGGA GGAGAAGACC ACCCCCAGCC AGAAGCAGGA GCCCATTGAC  
 AAGGAGCTGT ACCCCCTGGC CTCCCTGAGG TCCCTGTTTG GCAACGACCC CTCTTCCAG  
 ATGGCTCCCA TCTCCCCCAT TGAGACTGTG CCTGTGAAGC TGAAGCCTGG CATGGATGGC  
 CCAAGGTGA AGCAGTGGCC CTGACTGAG GAGAAGATCA AGGCCCTGGT GGAAATCTGC  
 ACTGAGATGG AGAAGGAGGG CAAAATCTCC AAGATTGGCC CCGAGAACCC CTACAACACC  
 CCTGTGTTTG CCATCAAGAA GAAGGACTCC ACCAAGTGA GGAAGCTGGT GGACTTCAGG  
 GAGCTGAACA AGAGGACCCA GGACTTCTGG GAGGTGCAGC TGGGCATCCC CCACCCCGCT  
 GGCTGAAGA AGAAGAAGTC TGTGACTGTG CTGGCTGTGG GGGATGCCTA CTTCTCTGTG  
 CCCCTGGATG AGGACTTCAG GAAGTACACT GCCTTCACCA TCCCCTCCAT CAACAATGAG  
 ACCCCTGGCA TCAGGTACCA GTACAATGTG CTGCCCCAGG GCTGGAAGGG CTCCCCTGCC  
 ATCTTCCAGT CCTCCATGAC CAAGATCCTG GAGCCCTTCA GGAAGCAGAA CCCTGACATT  
 GTGATCTACC AGTACATGGC TGCCCTGTAT GTGGGCTCTG ACCTGGAGAT TGGGCAGCAC  
 AGGACCAAGA TTGAGGAGCT GAGGCAGCAC CTGCTGAGGT GGGGCCTGAC CACCCCTGAC  
 AAGAAGCACC AGAAGGAGCC CCCCTTCTCTG TGGATGGGCT ATGAGCTGCA CCCCAGACAAG  
 TGGACTGTGC AGCCCATTTG GCTGCCCTGAG AAGGACTCCT GGACTGTGAA TGACATCCAG  
 AAGCTGGTGG GCAAGCTGAA CTGGGCCTCC CAAATCTACC CTGGCATCAA GGTGAGGCAG  
 CTGTGCAAGC TGCTGAGGGG CACCAAGGCC CTGACTGAGG TGATCCCCCT GACTGAGGAG  
 GCTGAGCTGG AGCTGGCTGA GAACAGGGAG ATCCTGAAGG AGCCTGTGCA TGGGGTGTA

FIGURE 33B

TATGACCCCT CCAAGGACCT GATTGCTGAG ATCCAGAAGC AGGGCCAGGG CCAGTGGACC  
TACCAAATCT ACCAGGAGCC CTTCAAGAAC CTGAAGACTG GCAAGTATGC CAGGATGAGG  
GGGGCCCACA CCAATGATGT GAAGCAGCTG ACTGAGGCTG TGCAGAAGAT CACCACTGAG  
TCCATTGTGA TCTGGGGCAA GACCCCAAG TTCAAGCTGC CCATCCAGAA GGAGACCTGG  
GAGACCTGGT GGA CTGAGTA CTGGCAGGCC ACCTGGATCC CTGAGTGGGA GTTTGTGAAC  
ACCCCCCCC TGGTGAAGCT GTGGTACCAG CTGGAGAAGG AGCCCATTTGT GGGGGCTGAG  
ACCTTCTATG TGGCTGGGGC TGCCAACAGG GAGACCAAGC TGGGCAAGGC TGGCTATGTG  
ACCAACAGGG GCAGGCAGAA GGTGGTGACC CTGACTGACA CCACCAACCA GAAGACTGCC  
CTCCAGGCCA TCTACCTGGC CCTCCAGGAC TCTGGCCTGG AGGTGAACAT TGTGACTGCC  
TCCCAGTATG CCCTGGGCAT CATCCAGGCC CAGCCTGATC AGTCTGAGTC TGAGCTGGTG  
AACCAGATCA TTGAGCAGCT GATCAAGAAG GAGAAGGTGT ACCTGGCCTG GGTGCCTGCC  
CACAAGGGCA TTGGGGGCAA TGAGCAGGTG GACAAGCTGG TGTCTGCTGG CATCAGGAAG  
GTGCTGTTCC TGGATGGCAT TGACAAGGCC CAGGATGAGC ATGAGAAGTA CCACTCCAAC  
TGGAGGGCTA TGGCCTCTGA CTTCAACCTG CCCCCTGTGG TGGCTAAGGA GATTGTGGCC  
TCCTGTGACA AGTGCCAGCT GAAGGGGGAG GCCATGCATG GGCAGGTGGA CTGCTCCCCT  
GGCATCTGGC AGCTGGCCTG CACCCACCTG GAGGGCAAGG TGATCCTGGT GGCTGTGCAT  
GTGGCCTCCG GCTACATTGA GGCTGAGGTG ATCCCTGCTG AGACAGGCCA GGAGACTGCC  
TACTTCCTGC TGAAGCTGGC TGGCAGGTGG CCTGTGAAGA CCATCCACAC TGCCAATGGC  
TCCAACCTCA CTGGGGCCAC AGTGAGGGCT GCCTGCTGGT GGGCTGGCAT CAAGCAGGAG  
TTTGGCATCC CCTACAACCC CCAGTCCCAG GGGGTGGTGG CCTCCATGAA CAAGGAGCTG  
AAGAAGATCA TTGGGCAGGT GAGGGACCAG GCTGAGCACC TGAAGACAGC TGTGCAGATG  
GCTGTGTTCA TCCACAACCT CAAGAGGAAG GGGGGCATCG GGGGCTACTC CGCTGGGGAG  
AGGATTGTGG ACATCATTCG CACAGACATC CAGACCAAGG AGCTCCAGAA GCAGATCACC  
AAGATCCAGA ACTTCAGGGT GTACTACAGG GACTCCAGGA ACCCCCTGTG GAAGGGCCCT  
GCCAAGCTGC TGTGGAAGGG GGAGGGGGCT GTGGTGATCC AGGACAACCTC TGACATCAAG  
GTGGTGCCCA GGAGGAAGGC CAAGATCATC AGGGACTATG GCAAGCAGAT GGCTGGGGAT  
GACTGTGTGG CCTCCAGGCA GGATGAGGAC TAA

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FIGURE 34A

Met Gly Ala Arg Ala Ser Val Leu Ser Gly Gly Glu Leu Asp Lys Trp Glu Lys  
 Ile Arg Leu Arg Pro Gly Gly Lys Lys Lys Tyr Lys Leu Lys His Ile Val Trp  
 Ala Ser Arg Glu Leu Glu Arg Phe Ala Val Asn Pro Gly Leu Leu Glu Thr Ser  
 Glu Gly Cys Arg Gln Ile Leu Gly Gln Leu Gln Pro Ser Leu Gln Thr Gly Ser  
 Glu Glu Leu Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys Val His Gln  
 Lys Ile Asp Val Lys Asp Thr Lys Glu Ala Leu Glu Lys Ile Glu Glu Glu Gln  
 Asn Lys Ser Lys Lys Lys Ala Gln Gln Ala Ala Ala Gly Thr Gly Asn Ser Ser  
 Gln Val Ser Gln Asn Tyr Pro Ile Val Gln Asn Leu Gln Gly Gln Met Val His  
 Gln Ala Ile Ser Pro Arg Thr Leu Asn Ala Trp Val Lys Val Val Glu Glu Lys  
 Ala Phe Ser Pro Glu Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala Thr  
 Pro Gln Asp Leu Asn Thr Met Leu Asn Thr Val Gly Gly His Gln Ala Ala Met  
 Gln Met Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala Glu Trp Asp Arg Leu His  
 Pro Val His Ala Gly Pro Ile Ala Pro Gly Gln Met Arg Glu Pro Arg Gly Ser  
 Asp Ile Ala Gly Thr Thr Ser Thr Leu Gln Glu Gln Ile Gly Trp Met Thr Asn  
 Asn Pro Pro Ile Pro Val Gly Glu Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu  
 Asn Lys Ile Val Arg Met Tyr Ser Pro Thr Ser Ile Leu Asp Ile Arg Gln Gly  
 Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala  
 Glu Gln Ala Ser Gln Glu Val Lys Asn Trp Met Thr Glu Thr Leu Leu Val Gln  
 Asn Ala Asn Pro Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro Ala Ala Thr  
 Leu Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly Pro Gly His Lys Ala  
 Arg Val Leu Ala Glu Ala Met Ser Gln Val Thr Asn Ser Ala Thr Ile Met Met  
 Gln Arg Gly Asn Phe Arg Asn Gln Arg Lys Thr Val Lys Cys Phe Asn Cys Gly  
 Lys Val Gly His Ile Ala Lys Asn Cys Arg Ala Pro Arg Lys Lys Gly Cys Trp  
 Lys Cys Gly Lys Glu Gly His Gln Met Lys Asp Cys Asn Glu Arg Gln Ala Asn  
 Phe Leu Gly Lys Ile Trp Pro Ser His Lys Gly Arg Pro Gly Asn Phe Leu Gln  
 Ser Arg Pro Glu Pro Thr Ala Pro Pro Glu Glu Ser Phe Arg Phe Gly Glu Glu  
 Lys Thr Thr Pro Ser Gln Lys Gln Glu Pro Ile Asp Lys Glu Leu Tyr Pro Leu  
 Ala Ser Leu Arg Ser Leu Phe Gly Asn Asp Pro Ser Ser Gln Met Ala Pro Ile  
 Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys  
 Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys  
 Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr  
 Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu  
 Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu  
 Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Ala  
 Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr  
 Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr  
 Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met  
 Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln  
 Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr  
 Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp  
 Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro

FIGURE 34B

Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val  
Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro  
Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr  
Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu  
Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile  
Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu  
Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr  
Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile  
Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp  
Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe  
Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile  
Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu  
Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr  
Asp Thr Thr Asn Gln Lys Thr Ala Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp  
Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile  
Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln  
Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile  
Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu  
Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn  
Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile  
Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val  
Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu Gly Lys Val  
Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro  
Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp  
Pro Val Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val  
Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn  
Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile  
Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val  
Phe Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly Glu  
Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln  
Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu  
Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln  
Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp  
Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp  
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(54) Title: ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIV-1 GAG, POL, NEF AND MODIFICATIONS

WO 02/022080 A3

(57) Abstract: First generation adenoviral vectors and associated recombinant adenovirus-based HIV vaccines which show enhanced stability and growth properties and greater cellular-mediated immunity are described within this specification. These adenoviral vectors are utilized to generate and produce through cell culture various adenoviral-based HIV-1 vaccines which contain HIV-1 gag, HIV-1 pol and/or HIV-1 nef polynucleotide pharmaceutical products, and biologically relevant modifications thereof. These adenovirus vaccines, when directly introduced into living vertebrate tissue, preferably a mammalian host such as a human or a non-human mammal of commercial or domestic veterinary importance, express the HIV-1 Gag, Pol and/or Nef protein or biologically modification thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding HIV-1 Gag, encoding codon optimized HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNase H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The adenoviral vaccines of the present invention, when administered alone or in a combined modality regime, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.





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*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

**TITLE OF THE INVENTION**

**ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING  
CODON OPTIMIZED HIV-1-GAG, POL, NEF AND MODIFICATIONS**

**5 CROSS-REFERENCE TO RELATED APPLICATIONS**

This application claims the benefit, under 35 U.S.C. §119(e), of U.S. provisional applications 60/233,180, 60/279,056, and Attorney Docket 20867PV2 (serial number unassigned), filed September 15, 2000, March 27, 2001, and September 7, 2001, respectively.

10

**STATEMENT REGARDING FEDERALLY-SPONSORED R&D**

Not Applicable

**REFERENCE TO MICROFICHE APPENDIX**

15

Not Applicable

**FIELD OF THE INVENTION**

The present invention relates to recombinant, replication-deficient first generation adenovirus vaccines found to exhibit enhanced growth properties and greater cellular-mediated immunity as compared to other replication-deficient vectors. The invention also relates to the associated first generation adenoviral vectors described herein, which, through the incorporation of additional 5' adenovirus sequence, enhance large scale production efficiency of the recombinant, replication-defective adenovirus described herein. Another aspect of the instant-invention is the surprising discovery that the intron A portion of the human cytomegalovirus (hCMV) promoter constitutes a region of instability in adenoviral vector constructs. Removal of this region from adenoviral expression constructs results in greatly improved vector stability. Therefore, improved vectors expressing a transgene under the control of an intron A-deleted CMV promoter constitute a further aspect of this invention. These adenoviral vectors are useful for generating recombinant adenovirus vaccines against human immunodeficiency virus (HIV). In particular, the first generation adenovirus vectors disclosed herein are utilized to construct and generate adenovirus-based HIV-1 vaccines which contain HIV-1 Gag, HIV-1 Pol and/or HIV-1 Nef polynucleotide pharmaceutical products, and biologically active modifications thereof. Host administration of the recombinant, replication-deficient adenovirus vaccines described herein results in expression of HIV-1 Gag, HIV-1- Pol and/or Nef protein or

immunologically relevant modifications thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding codon optimized HIV-1 Gag, HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNase H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef, and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The HIV adenovirus vaccines of the present invention, when administered alone or in a combined modality and/or prime/boost regimen, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.

#### BACKGROUND OF THE INVENTION

Human Immunodeficiency Virus-1 (HIV-1) is the etiological agent of acquired human immune deficiency syndrome (AIDS) and related disorders. HIV-1 is an RNA virus of the Retroviridae family and exhibits the 5' LTR-*gag-pol-env*-LTR 3' organization of all retroviruses. The integrated form of HIV-1, known as the provirus, is approximately 9.8 Kb in length. Each end of the viral genome contains flanking sequences known as long terminal repeats (LTRs). The HIV genes encode at least nine proteins and are divided into three classes; the major structural proteins (Gag, Pol, and Env), the regulatory proteins (Tat and Rev); and the accessory proteins (Vpu, Vpr, Vif and Nef).

The *gag* gene encodes a 55-kilodalton (kDa) precursor protein (p55) which is expressed from the unspliced viral mRNA and is proteolytically processed by the HIV protease, a product of the *pol* gene. The mature p55 protein products are p17 (matrix), p24 (capsid), p9 (nucleocapsid) and p6.

The *pol* gene encodes proteins necessary for virus replication; a reverse transcriptase, a protease, integrase and RNase H. These viral proteins are expressed as a Gag-Pol fusion protein, a 160 kDa precursor protein which is generated via a ribosomal frame shifting. The viral encoded protease proteolytically cleaves the Pol polypeptide away from the Gag-Pol fusion and further cleaves the Pol polypeptide to the mature proteins which provide protease (Pro, P10), reverse transcriptase (RT, P50), integrase (IN, p31) and RNase H (RNase, p15) activities.

The *nef* gene encodes an early accessory HIV protein (Nef) which has been shown to possess several activities such as down regulating CD4 expression, disturbing T-cell activation and stimulating HIV infectivity.

5 The *env* gene encodes the viral envelope glycoprotein that is translated as a 160-kilodalton (kDa) precursor (gp160) and then cleaved by a cellular protease to yield the external 120-kDa envelope glycoprotein (gp120) and the transmembrane 41-kDa envelope glycoprotein (gp41). Gp120 and gp41 remain associated and are displayed on the viral particles and the surface of HIV-infected cells.

10 The *tat* gene encodes a long form and a short form of the Tat protein, a RNA binding protein which is a transcriptional transactivator essential for HIV-1 replication.

The *rev* gene encodes the 13 kDa Rev protein, a RNA binding protein. The Rev protein binds to a region of the viral RNA termed the Rev response element (RRE). The Rev protein promotes transfer of unspliced viral RNA from the nucleus to the cytoplasm. The Rev protein is required for HIV late gene expression and in turn, HIV replication.

20 Gp120 binds to the CD4/chemokine receptor present on the surface of helper T-lymphocytes, macrophages and other target cells in addition to other co-receptor molecules. X4 (macrophage tropic) virus show tropism for CD4/CXCR4 complexes while a R5 (T-cell line tropic) virus interacts with a CD4/CCR5 receptor complex. After gp120 binds to CD4, gp41 mediates the fusion event responsible for virus entry. The virus fuses with and enters the target cell, followed by reverse transcription of its single stranded RNA genome into the double-stranded DNA via a RNA dependent DNA polymerase. The viral DNA, known as provirus, enters the cell nucleus, where the viral DNA directs the production of new viral RNA within the nucleus, expression of early and late HIV viral proteins, and subsequently the production and cellular release of new virus particles. Recent advances in the ability to detect viral load within the host shows that the primary infection results in an extremely high generation and tissue distribution of the virus, followed by a steady state level of virus (albeit through a continual viral production and turnover during this phase), leading ultimately to another burst of virus load which leads to the onset of clinical AIDS. Productively infected cells have a half life of several days, whereas chronically or latently infected cells have a 3-week half life, followed by non-productively infected cells which have a long half life (over 100 days) but do not significantly contribute to day to day viral loads seen throughout the course of disease.

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Destruction of CD4 helper T lymphocytes, which are critical to immune defense, is a major cause of the progressive immune dysfunction that is the hallmark of HIV infection. The loss of CD4 T-cells seriously impairs the body's ability to fight most invaders, but it has a particularly severe impact on the defenses against viruses, fungi, parasites and certain bacteria, including mycobacteria.

Effective treatment regimens for HIV-1 infected individuals have become available recently. However, these drugs will not have a significant impact on the disease in many parts of the world and they will have a minimal impact in halting the spread of infection within the human population. As is true of many other infectious diseases, a significant epidemiologic impact on the spread of HIV-1 infection will only occur subsequent to the development and introduction of an effective vaccine. There are a number of factors that have contributed to the lack of successful vaccine development to date. As noted above, it is now apparent that in a chronically infected person there exists constant virus production in spite of the presence of anti-HIV-1 humoral and cellular immune responses and destruction of virally infected cells. As in the case of other infectious diseases, the outcome of disease is the result of a balance between the kinetics and the magnitude of the immune response and the pathogen replicative rate and accessibility to the immune response. Pre-existing immunity may be more successful with an acute infection than an evolving immune response can be with an established infection. A second factor is the considerable genetic variability of the virus. Although anti-HIV-1 antibodies exist that can neutralize HIV-1 infectivity in cell culture, these antibodies are generally virus isolate-specific in their activity. It has proven impossible to define serological groupings of HIV-1 using traditional methods. Rather, the virus seems to define a serological "continuum" so that individual neutralizing antibody responses, at best, are effective against only a handful of viral variants. Given this latter observation, it would be useful to identify immunogens and related delivery technologies that are likely to elicit anti-HIV-1 cellular immune responses. It is known that in order to generate CTL responses antigen must be synthesized within or introduced into cells, subsequently processed into small peptides by the proteasome complex, and translocated into the endoplasmic reticulum/Golgi complex secretory pathway for eventual association with major histocompatibility complex (MHC) class I proteins. CD8<sup>+</sup> T lymphocytes recognize antigen in association with class I MHC via the T cell receptor (TCR) and the CD8 cell surface protein. Activation of naive CD8<sup>+</sup> T cells into activated effector or memory cells generally requires both TCR engagement of antigen as described above as well as engagement of costimulatory proteins. Optimal

induction of CTL responses usually requires "help" in the form of cytokines from CD4<sup>+</sup> T lymphocytes which recognize antigen associated with MHC class II molecules via TCR and CD4 engagement.

European Patent Applications 0 638 316 (Published February 15, 1995) and 0 586 076 (Published March 9, 1994), (both assigned to American Home Products Corporation) describe replicating adenovirus vectors carrying an HIV gene, including *env* or *gag*. Various treatment regimens were used with chimpanzees and dogs, some of which included booster adenovirus or protein plus alum treatments.

Replication-defective adenoviral vectors harboring deletions in the E1 region are known, and recent adenoviral vectors have incorporated the known packaging repeats into these vectors; e.g., see EP 0 707 071, disclosing, *inter alia*, an adenoviral vector deleted of E1 sequences from base pairs 459 to 3328; and U.S. Patent No. 6,033,908, disclosing, *inter alia*, an adenoviral vector deleted of base pairs 459-3510. The packaging efficiency of adenovirus has been taught to depend on the number of incorporated individual A (packaging) repeats; *see, e.g.*, Gräble and Hearing, 1990 *J. Virol.* 64(5):2047-2056; Gräble and Hearing, 1992 *J. Virol.* 66(2):723-731.

Larder, et al., (1987, *Nature* 327: 716-717) and Larder, et al., (1989, *Proc. Natl. Acad. Sci.* 86: 4803-4807) disclose site specific mutagenesis of HIV-1 RT and the effect such changes have on *in vitro* activity and infectivity related to interaction with known inhibitors of RT.

Davies, et al. (1991, *Science* 252:, 88-95) disclose the crystal structure of the RNase H domain of HIV-1 Pol.

Schatz, et al. (1989, *FEBS Lett.* 257: 311-314) disclose that mutations Glu478Gln and His539Phe in a complete HIV-1 RT/RNase H DNA fragment results in defective RNase activity without effecting RT activity.

Mizrahi, et al. (1990, *Nucl. Acids. Res.* 18: pp. 5359-5353) disclose additional mutations Asp443Asn and Asp498Asn in the RNase region of the *pol* gene which also results in defective RNase activity. The authors note that the Asp498Asn mutant was difficult to characterize due to instability of this mutant protein.

Leavitt, et al. (1993, *J. Biol. Chem.* 268: 2113-2119) disclose several mutations, including a Asp64Val mutation, which show differing effect on HIV-1 integrase (IN) activity.

Wiskerchen, et al. (1995, *J. Virol.* 69: 376-386) disclose single and double mutants, including mutation of aspartic acid residues which effect HIV-1 IN and viral replication functions.

It would be of great import in the battle against AIDS to produce a prophylactic- and/or therapeutic-based HIV vaccine which generates a strong cellular immune response against an HIV infection. The present invention addresses and meets these needs by disclosing a class of adenovirus vaccines which, upon host administration, express codon optimized and modified versions of the HIV-1 genes, *gag*, *pol* and *nef*. These recombinant, replication-defective adenovirus vaccines may be administered to a host, such as a human, alone or as part of a combined modality regimen and/or prime-boost vaccination regimen with components of the present invention and/or a distinct viral HIV DNA vaccine, non-viral HIV DNA vaccine, HIV subunit vaccine, an HIV whole killed vaccine and/or a live attenuated HIV vaccine.

#### SUMMARY OF THE INVENTION

The present invention relates to enhanced replication-defective recombinant adenovirus vaccine vectors and associated recombinant, replication-deficient adenovirus vaccines which encode various forms of HIV-1 Gag, HIV-1 Pol, and/or HIV-1 Nef, including immunologically relevant modifications of HIV-1 Gag, HIV-1 Pol and HIV-1 Nef. The adenovirus vaccines of the present invention express HIV antigens and provide for improved cellular-mediated immune responses upon host administration. Potential vaccinees include but are not limited to primates and especially humans and non-human primates, and also include any non-human mammal of commercial or domestic veterinary importance. An effect of the improved recombinant adenovirus-based vaccines of the present invention should be a lower transmission rate to previously uninfected individuals (i.e., prophylactic applications) and/or reduction in the levels of the viral loads within an infected individual (i.e., therapeutic applications), so as to prolong the asymptomatic phase of HIV-1 infection. In particular, the present invention relates to adenoviral-based vaccines which encode various forms of codon optimized HIV-1 Gag (including but in no way limited to p55 versions of codon optimized full length (FL) Gag and tPA-Gag fusion proteins), HIV-1 Pol, HIV-1 Nef, and selected modifications of immunological relevance. The administration, intracellular delivery and expression of these adenovirus vaccines elicit a host CTL and Th response. The preferred replication-defective recombinant adenoviral vaccine vectors include but are not limited to synthetic DNA molecules which (1) encode codon optimized versions of wild type HIV-1 Gag; (2) encode codon optimized versions of HIV-1 Pol; (3) encode codon optimized versions of HIV-1 Pol fusion proteins; (4) encode codon optimized versions of modified HIV-1 Pol proteins and fusion proteins, including but not limited

to *pol* modifications involving residues within the catalytic regions responsible for RT, RNase and IN activity within the host cell; (5) encode codon optimized versions of wild type HIV-1 Nef; (6) codon optimized versions of HIV-1 Nef fusion proteins; and/or (7) codon optimized versions of HIV-1 Nef derivatives, including but not limited to *nef* modifications involving introduction of an amino-terminal leader sequence, removal of an amino-terminal myristylation site and/or introduction of dileucine motif mutations. The Nef-based fusion and modified proteins, disclosed within this specification and expressed from an adenoviral-based vector vaccine this specification, may possess altered trafficking and/or host cell function while retaining the ability to be properly presented to the host MHC I complex and in turn elicit a host CTL and Th response. Examples of HIV-1 Gag, Pol and/or Nef fusion proteins include but are not limited to fusion of a leader or signal peptide at the NH<sub>2</sub>-terminal portion of the viral antigen coding region. Such a leader peptide includes but is not limited to a tPA leader peptide.

The adenoviral vector utilized in construction of the HIV-1 Gag-, HIV-1 Pol- and/or HIV-1 Nef- based vaccines of the present invention may comprise any replication-defective adenoviral vector which provides for enhanced genetic stability of the recombinant adenoviral genome through large scale production and purification of the recombinant virus. In other words, an HIV-1 Gag-, Pol- or Nef-based adenovirus vaccine of the present invention is a purified recombinant, replication-defective adenovirus which is shown to be genetically stable through multiple passages in cell culture and remains so during large scale production and purification procedures. Such a recombinant adenovirus vector and harvested adenovirus vaccine lends itself to large scale dose filling and subsequent worldwide distribution procedures which will be demanded of an efficacious monovalent or multivalent HIV vaccine. The present invention meets this basic requirement with description of a replication-defective adenoviral vector and vectors derived therefrom, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 of the wildtype adenovirus genome. A preferred embodiment of the instant invention comprises base pairs 1-450 of a wildtype adenovirus. In other preferred embodiments, the replication-defective adenoviral vector has, in addition thereto, a region 3' to the E1-deleted region comprising base pairs 3511-3523. Basepairs 342-450 (more particularly, 400-450) constitute an extension of the 5'-region of previously disclosed vectors carrying viral antigens, particularly HIV antigens (see, e.g., PCT International Application PCT/US00/18332, published



January 11, 2001 (WO 01/02067), which claims priority to U.S. Provisional Application Serial Nos. 60/142,631 and 60/148,981, filed 7/6/1999 and 8/13/1999, respectively; these documents herein incorporated by reference. Applicants have found that extending the 5' region further into the E1 gene into the disclosed vaccine  
5 vectors incorporated elements found to be important in optimizing the packaging of the virus.

As compared to previous vectors not comprising basepairs from about 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 of the wildtype adenovirus genome, vectors comprising the above region exhibited enhanced  
10 growth characteristics, with approximately 5-10 fold greater amplification rates, a more potent virus effect, allowing lower doses of virus to be used to generate equivalent immunity; and a greater cellular-mediated immune response than replication-deficient vectors not comprising this region (basepairs 1-450). Even more important, adenoviral constructs derived therefrom are very stable genetically in  
15 large-scale production, particularly those comprising an expression cassette under the control of a hCMV promoter devoid of intron A. This is because Applicants have surprisingly found that the intron A portion of the hCMV promoter constituted a region of instability when employed in adenoviral vectors. Applicants have, therefore, identified an enhanced adenoviral vector which is particularly suited for use  
20 in gene therapy and nucleotide-based vaccine vectors which, favorably, lends itself to large scale propagation.

A preferred embodiment of this invention is a replication-defective adenoviral vector in accordance with the above description wherein the gene is inserted in the form of a gene expression cassette comprising (a) a nucleic acid encoding a protein or  
25 biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and, (c) a transcription terminator.

In preferred embodiments, the E1 gene, other than that contained within basepairs 1-450 or, alternatively, that contained within base pairs 1-450 and 3511-  
30 3523 has been deleted from the adenoviral vector, and the gene expression cassette has replaced the deleted E1 gene. In other preferred embodiments, the replication defective adenovirus genome does not have a functional E3 gene, or the E3 gene has been deleted. Most preferably, the E3 region is present within the adenoviral genome. Further preferred embodiments are wherein the gene expression cassette is in an E1  
35 anti-parallel (transcribed in a 3' to 5' direction relative to the vector backbone)

orientation or, more preferably, an E1 parallel (transcribed in a 5' to 3' direction relative to the vector backbone) orientation.

Further embodiments relate to a shuttle plasmid vector comprising: an adenoviral portion and a plasmid portion, wherein said adenovirus portion comprises:

- 5 a) a replication defective adenovirus genome, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 (preferably, 1-450) of the wildtype adenovirus genome and, preferably, in addition thereto, basepairs 3511-3523 of a wildtype adenovirus sequence; and b) a gene  
10 expression cassette comprising: (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and (c) a transcription terminator and/or a polyadenylation site.

- Other aspects of this invention include a host cell comprising said adenoviral  
15 vectors and/or said shuttle plasmid vectors; vaccine compositions comprising said vectors; and methods of producing the vectors comprising (a) introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and (b) harvesting the resultant adenoviral vectors.

- To this end, the present invention particularly relates to harvested  
20 recombinant, replication defective virus derived from a host cell, such as but not limited to 293 cells or PER.C6<sup>®</sup> cells, including but not limited to harvested virus related to any of the MRKAd5 vector backbones, with or without an accompanying transgene, including but not limited to the HIV-1 antigens described herein. An HIV-1 vaccine is represented by any harvested, recombinant adenovirus material  
25 which expresses any one or more of the HIV-1 antigens disclosed herein. This harvested material may then be purified, formulated and stored prior to host administration.

- Another aspect of this invention is a method of generating a cellular immune response against a protein in an individual comprising administering to the individual  
30 an adenovirus vaccine vector comprising:

- a) a recombinant, replication defective adenoviral vector, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting adenovirus packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 (preferably, 1-450) and, preferably in addition thereto,  
35 base pairs 3511-3523 of a wildtype adenovirus sequence, and,

b) a gene expression cassette comprising: (i) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (ii) a heterologous promoter operatively linked to the nucleic acid of part a); and (iii) a transcription terminator and/or a polyadenylation site.

5           In view of the efficacious nature of the adenoviral and/or DNA plasmid vaccines described herein, the present invention relates to all methodology regarding administration of one or more of these adenoviral and/or DNA plasmid vaccines to provide effective immunoprophylaxis, to prevent establishment of an HIV-1 infection following exposure to this virus, or as a post-HIV infection therapeutic vaccine to  
10       mitigate the acute HIV-1 infection so as to result in the establishment of a lower virus load with beneficial long term consequences. As discussed herein, such a treatment regimen may include a monovalent or multivalent composition, various combined modality applications, and/or a prime/boost regimen to as to optimize antigen expression and a concomitant cellular-mediated and/or humoral immune response  
15       upon inoculation into a living vertebrate tissue. Therefore, the present invention provides for methods of using the adenoviral and/or DNA plasmid vaccines disclosed herein within the various parameters disclosed herein as well as any additional parameters known in the art, which, upon introduction into mammalian tissue induces intracellular expression of the gag, pol and/or nef-based vaccines.

20           To this end, the present invention relates in part to methods of generating a cellular immune response in a vaccinee, preferably a human vaccinee, wherein the individual is given more than one administration of adenovirus vaccine vector, and it may be given in a regimen accompanied by the administration of a plasmid vaccine. The plasmid vaccine (also referred to herein as a "DNA plasmid vaccine" or "vaccine  
25       plasmid" comprises a nucleic acid encoding a protein or an immunologically relevant portion thereof, a heterologous promoter operably linked to the nucleic acid sequence, and a transcription terminator or a polyadenylation signal (such as bGH or SPA, respectively). There may be a predetermined minimum amount of time separating the administrations. The individual can be given a first dose of plasmid vaccine, and then  
30       a second dose of plasmid vaccine. Alternatively, the individual may be given a first dose of adenovirus vaccine, and then a second dose of adenovirus vaccine. In other embodiments, the plasmid vaccine is administered first, followed after a time by administration of the adenovirus vaccine. Conversely, the adenovirus vaccine may be administered first, followed by administration of plasmid vaccine after a time. In  
35       these embodiments, an individual may be given multiple doses of the same adenovirus serotype in either viral vector or plasmid form, or the virus may be of

differing serotypes. In the alternative, a viral antigen of interest can be first delivered via a viral vaccine other than an adenovirus-based vaccine, and then followed with the adenoviral vaccine disclosed. Alternative viral vaccines include but are not limited to pox virus and venezuelan equine encephalitis virus.

5       The present invention also relates to multivalent adenovirus vaccine compositions which comprise Gag, Pol and Nef components described herein; see, e.g., Example 29 and Table 25. Such compositions will provide for an enhanced cellular immune response subsequent to host administration, particularly given the genetic diversity of human MHCs and of circulating virus. Examples, but not  
10       limitations, include MRKAd5-vector based multivalent vaccine compositions which provide for a divalent (i.e., gag and nef, gag and pol, or pol and nef components) or a trivalent vaccine (i.e., gag, pol and nef components) composition. Such a multivalent vaccine may be filled for a single dose or may consist of multiple inoculations of each individually filled component; and may in addition be part of a prime/boost regimen  
15       with viral or non-viral vector vaccines as introduced in the previous paragraph. To this end, preferred compositions are MRKAd5 adenovirus used in combination with multiple, distinct HIV antigen classes. Each HIV antigen class is subject to sequence manipulation, thus providing for a multitude of potential vaccine combinations; and such combinations are within the scope of the present invention. The utilization of  
20       such combined modalities vaccine formulation and administration increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

      The concept of a "combined modality" as disclosed herein also covers the alternative mode of administration whereby multiple HIV-1 viral antigens may be  
25       ligated into a proper shuttle plasmid for generation of a pre-adenoviral plasmid comprising multiple open reading frames. For example, a trivalent vector may comprise a gag-pol-nef fusion, in either a E3(-) or E3(+) background, preferably a E3 deleted backbone, or possibly a "2+1" divalent vaccine, such as a gag-pol fusion (i.e., codon optimized p55 gag and inactivated optimized pol; Example 29 and Table 25)  
30       within the same MRKAd5 backbone, with each open reading frame being operatively linked to a distinct promoter and transcription termination sequence. Alternatively, the two open reading frames may be operatively linked to a single promoter, with the open reading frames operatively linked by an internal ribosome entry sequence (IRES). Therefore, a multivalent vaccine delivered as a single, or possibly a second  
35       harvested recombinant, replication-deficient adenovirus is contemplated as part of the present invention.

Therefore, the adenoviral vaccines and plasmid DNA vaccines of this invention may be administered alone, or may be part of a prime and boost administration regimen. A mixed modality priming and booster inoculation scheme will result in an enhanced immune response, particularly if pre-existing anti-vector immune responses are present. This one aspect of this invention is a method of  
5 priming a subject with the plasmid vaccine by administering the plasmid vaccine at least one time, allowing a predetermined length of time to pass, and then boosting by administering the adenoviral vaccine. Multiple primings typically, 1-4, are usually employed, although more may be used. The length of time between priming and  
10 boost may typically vary from about four months to a year, but other time frames may be used. In experiments with rhesus monkeys, the animals were primed four times with plasmid vaccines, then were boosted 4 months later with the adenoviral vaccine. Their cellular immune response was notably higher than that of animals which had only received adenoviral vaccine. The use of a priming regimen may be particularly  
15 preferred in situations where a person has a pre-existing anti-adenovirus immune response.

It is an object of the present invention to provide for enhanced replication-defective recombinant adenoviral vaccine vector backbones. These recombinant adenoviral backbones may accept one or more transgenes, which may be  
20 passaged through cell culture for growth, amplification and harvest.

It is a further object to provide for enhanced replication-defective recombinant adenoviral vaccine vectors which encode various transgenes.

It is also an object of the present invention to provide for a harvested recombinant, replication-deficient adenovirus which shows enhanced growth and  
25 amplification rates while in combination with increased virus stability after continuous passage in cell culture. Such a recombinant adenovirus is particularly suited for use in gene therapy and nucleotide-based vaccine vectors which, favorably, lends itself to large scale propagation.

To this end, it is an object of the present invention to provide for (1) enhanced  
30 replication-defective recombinant adenoviral vaccine vectors as described herein which encode various forms of HIV-1 Gag, HIV-1 Pol, and/or HIV-1 Nef, including immunologically relevant modifications of HIV-1 Gag, HIV-1 Pol and HIV-1 Nef, and (2) harvested, purified recombinant replication-deficient adenovirus generated by passage of the adenoviral vectors of (1) through one or multiple passages through cell  
35 culture, including but not limited to passage through 293 cells or PER.C6® cells.

It is also an object of the present invention to provide for recombinant adenovirus harvested by one or multiple passages through cell culture. As relating to recombinant adenoviral vaccine vector, this recombinant virus is harvested and formulated for subsequent host administration.

5 It is also an object of the present invention to provide for replication-defective adenoviral vectors wherein at least one gene is inserted in the form of a gene expression cassette comprising (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and, (c) a transcription terminator.

10 It is also an object of the present invention to provide for a host cell comprising said adenoviral vectors and/or said shuttle plasmid vectors; vaccine compositions comprising said vectors; and methods of producing the vectors comprising (a) introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and (b) harvesting the resultant adenoviral vectors.

15 It is a further object of the present invention to provide for methods of generating a cellular immune response against a protein in an individual comprising administering to the individual an adenovirus vaccine vector comprising a) a replication defective adenoviral vector, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting packaging region from about base pair 1 to between from about base pair 20 342 (more preferably, 400) to about 450 (preferably, 1-450) and, preferably, 3511-3523 of a wildtype adenovirus sequence, and, b) a gene expression cassette comprising: (i) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (ii) a heterologous promoter operatively linked to the nucleic acid of part a); and (iii) a transcription terminator and/or a 25 polyadenylation site.

It is also an object of the present invention to provide various alternatives for vaccine administration regimes, namely administration of one or more adenoviral and/or DNA plasmid vaccines described herein to provide effective immunoprophylaxis for uninfected individuals or a therapeutic treatment for HIV 30 infected patients. Such processes include but are not limited to multivalent HIV-1 vaccine compositions, various combined modality regimes as well as various prime/boost alternatives. These methods of administration, relating to vaccine composition and/or scheduled administration, will increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a 35 single modality regimen.

As used throughout the specification and claims, the following definitions and abbreviations are used:

"HAART" refers to -- highly active antiretroviral therapy --.

"first generation" vectors are characterized as being replication-defective.

5 They typically have a deleted or inactivated E1 gene region, and preferably have a deleted or inactivated E3 gene region as well.

"AEX" refers to Anion Exchange chromatography.

"QPA" refers to Quick PCR-based Potency Assay.

"bps" refers to basepairs.

10 "s" or "str" denotes that the transgene is in the E1 parallel or "straight" orientation.

"PBMCs" refers to peripheral blood monocyte cells.

"FL" refers to full length.

"FLgag" refers to a full-length optimized gag gene, as shown in Figure 2.

15 "Ad5-Flgag" refers to an adenovirus serotype 5 replication deficient virus which carries an expression cassette which comprises a full length optimized gag gene under the control of a CMV promoter.

"Promoter" means a recognition site on a DNA strand to which an RNA polymerase binds. The promoter forms an initiation complex with RNA polymerase  
20 to initiate and drive transcriptional activity. The complex can be modified by activating sequences such as enhancers or inhibiting sequences such as silencers.

"Leader" means a DNA sequence at the 5' end of a structural gene which is transcribed along with the gene. This usually results a protein having an N-terminal peptide extension, often referred to as a pro-sequences.

25 "Intron" means a section of DNA occurring in the middle of a gene which does not code for an amino acid in the gene product. The precursor RNA of the intron is excised and is therefore not transcribed into mRNA not translated into protein.

"Immunologically relevant" or "biologically active" means (1) with regards to a viral protein, that the protein is capable, upon administration, of eliciting a  
30 measurable immune response within an individual sufficient to retard the propagation and/or spread of the virus and/or to reduce the viral load present within the individual; or (2) with regards to a nucleotide sequence, that the sequence is capable of encoding for a protein capable of the above.

"Cassette" refers to a nucleic acid sequence which is to be expressed, along  
35 with its transcription and translational control sequences. By changing the cassette, a vector can express a different sequence.

"bGHpA" refers to the bovine growth hormone transcription terminator/polyadenylation sequence.

"tPAgag" refers to a fusion between the leader sequence of the tissue plasminogen activator leader sequence and an optimized HIV gag gene, as exemplified in Figure 30A-B, whether in a DNA or adenovirus-based vaccine vector.

Where utilized, "IA" or "inact" refers to an inactivated version of a gene (e.g. IApol).

"MCS" is "multiple cloning site".

In general, adenoviral constructs, gene constructs are named by reference to the genes contained therein. For example:

"Ad5 HIV-1 gag", also referred to as the original HIV-1 gag adenoviral vector, is a vector containing a transgene cassette composed of a hCMV intron A promoter, the full length version of the human codon-optimized HIV-1 gag gene, and the bovine growth hormone polyadenylation signal. The transgene was inserted in the E1 antiparallel orientation in an E1 and E3 deleted adenovector.

"MRK Ad5 HIV-1 gag" also referred to as "MRKAd5gag" or "Ad5gag2" is an adenoviral vector taught herein which is deleted of E1, comprises basepairs 1-450 and 3511-3523, and has a human codon-optimized HIV-1 gene in an E1 parallel orientation under the control of a CMV promoter without intron A. The construct also comprises a bovine growth hormone polyadenylation signal.

"pV1JnsHIVgag", also referred to as "HIVFLgagPR9901", is a plasmid comprising the CMV immediate-early (IE) promoter and intron A, a full-length codon-optimized HIV gag gene, a bovine growth hormone-derived polyadenylation and transcriptional termination sequence, and a minimal pUC backbone.

"pV1JnsCMV(no intron)-FLgag-bGHpA" is a plasmid derived from pV1JnsHIVgag which is deleted of the intron A portion of CMV and which comprises the full length HIV gag gene. This plasmid is also referred to as "pV1JnsHIVgag-bGHpA", pV1Jns-hCMV-FL-gag-bGHpA" and "pV1JnsCMV(no intron) + FLgag + bGHpA".

"pV1JnsCMV(no intron)-FLgag-SPA" is a plasmid of the same composition as pV1JnsCMV(no intron)-FLgag-bGHpA except that the SPA termination sequence replaces that of bGHpA. This plasmid is also referred to as "pV1Jns-HIVgag-SPA" and pV1Jns-hCMV-FLgag-SPA".

"pdelE1sp1A" is a universal shuttle vector with no expression cassette (i.e., no promoter or polyA). The vector comprises wildtype adenovirus serotype 5 (Ad5) sequences from bp 1 to bp 341 and bp 3524 to bp 5798, and has a multiple cloning



site between the Ad5 sequences ending 341 bp and beginning 3524 bp. This plasmid is also referred to as the original Ad 5 shuttle vector.

"MRKpdelE1sp1A" or "MRKpdelE1(Pac/pIX/pack450)" or "MRKpdelE1(Pac/pIX/pack450)Cla1" is a universal shuttle vector with no expression cassette (i.e. no promoter or polyA) comprising wildtype adenovirus serotype 5 (Ad5) sequences from bp1 to bp450 and bp 3511 to bp 5798. The vector has a multiple cloning site between the Ad5 sequence ending 450 bp and beginning 3511 bp. This shuttle vector may be used to insert the CMV promoter and the bGHpA fragments in both the straight ("str". or E1 parallel) orientation or in the opposite (opp. or E1 antiparallel) orientation)

"MRKpdelE1(Pac/pIX/pack450)+CMVmin+bGHpA(str.)" is still another shuttle vector which is the modified vector that contains the CMV promoter (no intronA) and the bGHpA fragments. The expression unit containing the hCMV promoter (no intron A) and the bovine growth hormone polyadenylation signal has been inserted into the shuttle vector such that insertion of the gene of choice at a unique *Bgl*II site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1/E3+)Cla1 pre-plasmid. This shuttle vector, as shown in Figures 22 and 23, was used to insert the respective IAPol and G2A,LLAA nef genes directly into.

"MRKpdelE1-CMV(no intron)-FLgag-bGHpA" is a shuttle comprising Ad5 sequences from basepairs 1-450 and 3511-5798, with an expression cassette containing human CMV without intron A, the full-length human codon-optimized HIV gag gene and bovine growth hormone polyadenylation signal. This plasmid is also referred to as "MRKpdelE1 shuttle +hCMV-FL-gag-BGHpA"

"MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA" is an adenoviral vector comprising all Ad5 sequences except those nucleotides encompassing the E1 region (from 451-3510), a human CMV promoter without intron A, a full-length human codon-optimized HIV gag gene, and a bovine growth hormone polyadenylation signal. This vector is also referred to as "MRKpAdHVE3 + hCMV-FL-gag-BGHpA", "MRKpAd5HIV-1gag", "MRKpAd5gag", "pMRKAd5gag" or "pAd5gag2".

"pV1Jns-HIV-pol inact(opt)" or "pV1Jns-HIV IA pol (opt)" is the inactivated Pol gene (contained within SEQ ID NO:3) cloned into the *Bgl*II site of V1Jns (Figure 17A-C). As noted herein, various derivatives of HIV-1 pol may be cloned into a plasmid expression vector such as V1Jns or V1Jns-tPA, thus serving directly as DNA vaccine candidates or as a source for subcloning into an appropriate adenoviral vector.

"MRKpdel+hCMVmin+FL-pol+bGHpA(s)" is the "MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.)" shuttle mentioned above which contains the IA pol gene is the proper orientation. This shuttle vector is used in a bacterial recombination with MRKpAd(E1-/E3+)Cla1.

5 "MRKpAd+hCMVmin+FL-pol+bGHpA(S)E3+", also referred to herein as "pMRKAd5pol", is the pre-adenovirus plasmid which comprises a CMV-pol inact(opt)-pGHpA construct. The construction of this pre-adenovirus plasmid is shown in Figure 22.

10 "pV1Jns/nef (G2A,LLAA)" or "V1Jns/opt nef (G2A,LLAA)" comprises codon optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175 (SEQ ID NO:13; which comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662). This fragment is subcloned into the Bgl II site of V1Jns  
15 and/or V1Jns-tPA (Figures 16A-B). As noted above for HIV-1 pol, HIV-1 nef constructs may be cloned into a plasmid expression vector such as V1Jns or V1Jns-tPA, thus serving directly as DNA vaccine candidates or as a source for subcloning into an appropriate adenoviral vector.

20 "MRKpdelE1hCMVminFL-nefBGHpA(s)", also referred to herein as "pMRKAd5nef", is the pre-adenovirus plasmid which comprises a CMV-nef (G2A,LLAA) codon optimized sequence. The construction of this pre-adenovirus plasmid is shown in Figure 23.

#### BRIEF DESCRIPTION OF THE FIGURES

25 Figure 1 shows the original HIV-1 gag adenovector (Ad5HIV-1gag). This vector is disclosed in PCT International Application No. PCT/US00/18332 (WO 01/02607) filed July 3, 2000, claiming priority to U.S. Provisional Application Serial No. 60/142,631, filed July 6, 1999 and U.S. Application Serial No. 60/148,981, filed August 13, 1999, all three applications which are hereby incorporated by reference.

30 Figure 2 shows the nucleic acid sequence (SEQ ID NO: 29) of the optimized human HIV-1 gag open reading frame.

Figure 3 shows diagrammatically the new transgene constructs in comparison with the original gag transgene.

35 Figure 4 shows the modifications made to the original adenovector backbone in the generation of the novel vectors of the instant invention.

Figure 5 shows the virus mixing experiments that were carried out to determine the effects of the addition made to the packaging signal region (Expt. #1) and the E3 gene on viral growth (Expt. #2). The bars denote the region of modifications made to the E1 deletion.

5        Figure 6 shows an autoradiograph of viral DNA analysis following the viral mixing experiments described in Examples 6 and 7.

      Figures 7A, 7B and 7C are as follows: Figure 7A shows the hCMV-Flgag-bGHpA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Both E1 parallel and E1 antiparallel transgene orientation are represented. Figure 7B shows the hCMV-Flgag-SPA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Again, both E1 parallel and E1 antiparallel transgene orientation are represented. Figure 7C shows the mCMV-Flgag-bGHpA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Once again, both E1 parallel and E1 antiparallel transgene orientation are represented.

15        Figure 8A shows the experiment designed to test the effect of transgene orientation.

      Figure 8B shows the experiments designed to test the effect of polyadenylation signal.

20        Figure 9 shows viral DNA from the four adenoviral vectors tested (Example 12) at P5, following *Bst*E11 digestion.

      Figure 10 shows viral DNA analysis of passages 11 and 12 of MRKpAdHVE3, MRKAd5HIV-1gag, and MRKAd5HIV-1gagE3-.

25        Figure 11 shows viral DNA analysis (*Hind*III digestion) of passage 6 MRKpAdHVE3 and MRKAd5HIV-1gag used to initiate the viral competition study. The last two lanes are passage 11 analysis of duplicate passages of the competition study (each virus at MOI of 280 viral particles).

30        Figure 12 shows viral DNA analysis by *Hind* III digestion on high passage numbers for MRKAd5HIV-1gag in serum-containing media with collections made at specified times. The first lane shows the 1kb DNA size marker. The other lanes represent pre-plasmid control (digested with Pac1 and *Hind*III), MRKAd5HIV-1gag at P16, P19, and P21.

35        Figure 13 shows serum anti-p24 levels at 3 wks post i.m. immunization of balb/c mice (n=10) with varying doses of several Adgag constructs: (A) MRK Ad5 HIV-1 gag (through passage 5); (B) MRKAd5 hCMV-FLgag-bGHpA (E3-); (C) MRKAd5 hCMV-FLgag-SPA (E3+); (D) MRKAd5 mCMV-FLgag-bGHpA (E3+);

(E) research lot (293 cell-derived) of Ad5HIV-1 gag; and (F) clinical lot (Ad5gagFN0001) of Ad5HIV-1 gag. Reported are the geometric mean titers (GMT) for each cohort along with the standard error bars.

Figure 14 shows a restriction map of the pMRKAd5HIV-1gag vector.

5        Figures 15A-X illustrates the nucleotide sequence of the pMRKAd5HIV-1gag vector (SEQ ID NO:27.[coding] and SEQ ID NO:28 [non-coding]).

Figures 16A-B shows a schematic representation of DNA vaccine expression vectors V1Jns (A) and V1Jns-tPA (B), which are utilized for HIV-1 gag, pol and nef constructs in various DNA/viral vector combined modality regimens as disclosed  
10        herein.

Figures 17A-C shows the nucleotide (SEQ ID NO:3) and amino acid sequence (SEQ ID NO:4) of IA-Pol. Underlined codons and amino acids denote mutations, as listed in Table 1.

Figure 18 shows codon optimized nucleotide and amino acid sequences  
15        through the fusion junction of tPA-pol inact(opt) (contained within SEQ ID NOs: 7 and 8, respectively). The underlined portion represents the NH<sub>2</sub>-terminal region of IA-Pol.

Figures 19A-B show a nucleotide sequence comparison between wild type nef(jrfl) and codon optimized nef. The wild type nef gene from the jrfl isolate  
20        consists of 648 nucleotides capable of encoding a 216 amino acid polypeptide. WT, wild type sequence (SEQ ID NO:19); opt, codon-optimized sequence (contained within SEQ ID NO:1). The Nef amino acid sequence is shown in one-letter code (SEQ ID NO:2).

Figures 20A-C show nucleotide sequences at junctions between nef coding  
25        sequence and plasmid backbone of nef expression vectors V1Jns/nef (Figure 20A), V1Jns/nef(G2A,LLAA) (Figure 20B), V1Jns/tpanef (Figure 20C) and V1Jns/tpanef(LLAA) (Figure 20C, also). 5' and 3' flanking sequences of codon optimized nef or codon optimized nef mutant genes are indicated by bold/italic letters; nef and nef mutant coding sequences are indicated by plain letters. Also indicated (as  
30        underlined) are the restriction endonuclease sites involved in construction of respective nef expression vectors. V1Jns/tpanef and V1Jns/tpanef(LLAA) have identical sequences at the junctions.

Figure 21 shows a schematic presentation of nef and nef derivatives. Amino  
acid residues involved in Nef derivatives are presented. Glycine 2 and Leucine174  
35        and 175 are the sites involved in myristylation and dileucine motif, respectively. For both versions of the tpanef fusion genes, the putative leader peptide cleavage sites are

indicated with "\*", and a exogenous serine residue introduced during the construction of the mutants is underlined.

Figure 22 shows diagrammatically the construction of the pre-adenovirus plasmid construct, MRKAd5Pol.

5        Figure 23 shows diagrammatically the construction of the pre-adenovirus plasmid construct, MRKAd5Nef.

Figure 24 shows a comparison of clade B vs. clade C anti-gag T cell responses in clade B HIV-infected subjects.

10       Figure 25 shows a comparison of clade B vs. clade C anti-nef T cell responses in clade B HIV-infected subjects.

Figures 26A-AO illustrates the nucleotide sequence of the pMRKAd5HIV-1pol adenoviral vector (SEQ ID NO:32 [coding] and SEQ ID NO:33 [non-coding]), comprising the coding region of the inactivated pol gene (SEQ ID NO3).

15       Figures 27A-AM illustrates the nucleotide sequence of the pMRKAd5HIV-1 nef adenoviral vector (SEQ ID NO:34 [coding] and SEQ ID NO:35 [non-coding]), comprising the coding region of the inactivated pol gene (SEQ ID NO13).

Figure 28 shows the stability of MRKAd5 vectors comprising various promoter fragments (hCMV or mCMV) and terminations signals (bGH or SPA) in E3(+) or E3(-) backbones.

20       Figures 29A and B shows the anion-exchange HPLC viral particle concentrations of the freeze-thaw recovered cell associated virus at the 24, 36, 48, and 60 hpi time points (Figure 29A) and the timcourse QPA supernatant titers (Figure 29B) for MRKAd5gag, MRKAd5pol and MRKAd5nef.

25       Figure 30 shows the nucleotide sequence (SEQ ID NO:36) and amino acid sequence (SEQ ID NO:37) comprising the open reading frame of a representative tPA-gag fusion for use in the DNA and/or adenoviral vaccine disclosed herein.

30       Figure 31 shows the intracellular  $\gamma$ IFN staining of PBMCs collected at week 10 (post DNA prime) and week 30 (post Ad boost). The cells were stimulated overnight in the presence or absence of the gag peptide pool. They were subsequently stained using fluorescence-tagged anti-CD3, anti-CD8, anti-CD4, and anti- $\gamma$ IFN monoclonal antibodies. Each plot shows all CD3+ T cells which were segregated in terms of positive staining for surface CD8 and  $\gamma$ IFN production. The numbers in the upper right and lower right quadrants of each plot are the percentages of CD3+ cells that were CD8+ $\gamma$ IFN+ and CD4+ $\gamma$ IFN+, respectively.

35       Figure 32 shows a comparison of single-modality adenovirus immunization with DNA + adjuvant prime/adenovirus boost immunization.

Figures 33A-B show the nucleotide sequence (SEQ ID NO: 38) of the open reading frame for the gag-IAPol fusion of Example 29.

Figures 34A-B show the protein sequence (SEQ ID NO:39) of the gag-IAPol fusion frame.

5

#### DETAILED DESCRIPTION OF THE INVENTION

A novel replication-defective, or "first generation," adenoviral vector suitable for use in gene therapy or nucleotide-based vaccine vectors is described. This vector is at least partially deleted in E1 and comprises a wildtype adenovirus *cis*-acting packaging region from about base pair 1 to between about base pair 342 (more preferably, 400) to about 458 (preferably, 1-450) and, preferably, 3511-3523 of a wild-type adenovirus sequence. It has been found that a vector of this description possesses enhanced growth characteristics, with approximately 5-10 fold greater amplification rates, and is more potent allowing lower doses of virus to be used to generate equivalent immunity. The vector, furthermore, generates a harvested recombinant adenovirus which shows greater cellular-mediated immune responses than replication-deficient vectors not comprising this region (basepairs 342-450). Adenoviral constructs derived from these vectors are, further, very stable genetically, particularly those comprising a transgene under the control of a hCMV promoter devoid of intron A. Viruses in accordance with this description were passaged continually and analyzed; see Example 12. Each virus analyzed maintained its correct genetic structure. Analysis was also carried out under propagation conditions similar to that performed in large scale production. Again, the vectors were found to possess enhanced genetic stability; see Figure 12. Following 21 passages, the viral DNA showed no evidence of rearrangement, and was highly reproducible from one production lot to the next. The outcome of all relevant tests indicate that the adenoviral vector is extremely well suited for large-scale production of recombinant, replication-deficient adenovirus, as shown herein with the data associated with Figure 28.

A preferred adenoviral vector in accordance with this description is a vector comprising basepairs 1-450, which is deleted in E3. This vector can accommodate up to approximately 7,500 base pairs of foreign DNA inserts (or exogenous genetic material). Another preferred vector is one retaining E3 which comprises basepairs 1-450. A preferred vector of this description is an E3+ vector comprising basepairs 1-450 and 3511-3523. This vector, when deleted of the region spanning basepairs 451-3510, can accommodate up to approximately 4,850 base pairs of foreign DNA inserts

(or exogenous genetic material). The cloning capacities of the above vectors have been determined using 105% of the wildtype Ad5 sequence as the upper genome size limit.

Wildtype adenovirus serotype 5 is used as the basis for the specific basepair numbers provided throughout the specification. The wildtype adenovirus serotype 5 sequence is known and described in the art; *see*, Chroboczek *et al.*, 1992 *J. Virology* 186:280, which is hereby incorporated by reference. Accordingly, a particular embodiment of the instant invention is a vector based on the adenovirus serotype 5 sequence. One of skill in the art can readily identify the above regions in other adenovirus serotypes (e.g., serotypes 2, 4, 6, 12, 16, 17, 24, 31, 33, and 42), regions defined by basepairs corresponding to the above basepair positions given for adenovirus serotype 5. Accordingly, the instant invention encompasses all adenoviral vectors partially deleted in E1 comprising basepairs corresponding to 1-450 (particularly, 342-450) and, preferably, 3511-3523 of a wild-type adenovirus serotype 5 (Ad5) nucleic acid sequence. Particularly preferred embodiments of the instant invention are those derived from adenoviruses like Ad5 which are classified in subgroup C (e.g., Ad2).

Vectors in accordance with the instant invention are at least partially deleted in E1. Preferably the E1 region is completely deleted or inactivated. Most preferably, the region deleted of E1 is within basepairs 451-3510. It is to be noted that the extended 5' and 3' regions of the disclosed vectors are believed to effectively reduce the size of the E1 deletion of previous constructs without overlapping any part of the E1A/E1B gene present in the cell line used, i.e., the PER.C6<sup>®</sup> cell line transfected with base pairs 459-3510. Overlap of adenoviral sequences is avoided because of the possibility of recombination. One of ordinary skill in the art can certainly appreciate that the instant invention can, therefore, be modified if a different cell line transfected with a different segment of adenovirus DNA is utilized. For purposes of exemplification, a 5' region of base pairs 1 to up to 449 is more appropriate if a cell line is transfected with adenoviral sequence from base pairs 450-3510. This holds true as well in the consideration of segments 3' to the E1 deletion.

Preferred embodiments of the instant invention possess an intact E3 region (i.e., an E3 gene capable of encoding a functional E3). Alternate embodiments have a partially deleted E3, an inactivated E3 region, or a sequence completely deleted of E3. Applicants have found, in accordance with the instant invention, that virus comprising the E3 gene were able to amplify more rapidly compared with virus not comprising an E3 gene; see Figure 6 wherein a diagnostic CsCl band corresponding to the E3+ virus

tested (5,665 bp) was present in greater amount compared with the diagnostic band of 3,010 bp corresponding to the E3- virus. These results were obtained following a virus competition study involving mixing equal MOI ratio (1:1) of adenovectors both comprising the E3 gene and not comprising the E3 gene. This increased amplification capacity of the E3+ adenovectors was subsequently confirmed with growth studies; see Table 4A, wherein the E3+ virus exhibit amplification ratios of 470, 420 and 320 as compared with the 115 and 40-50 of the E3- constructs.

As stated above, vectors in accordance with the instant invention can accommodate up to approximately 4,850 base pairs of exogenous genetic material for an E3+ vector and approximately 7,500 base pairs for an E3- vector. Preferably, the insert brings the adenoviral vector as close as possible to a wild-type genomic size (e.g., for Ad5, 35,935 basepairs). It is well known that adenovirus amplifies best when they are close to their wild-type genomic size.

The genetic material can be inserted in an E1-parallel or an E1 anti-parallel orientation, as such is illustrated in Figure 7A, 7B, 7C and Figure 8A. Particularly preferred embodiments of the instant invention, have the insert in an E1-parallel orientation. Applicants have found, via competition experiments with plasmids containing transgenes in differing orientation (Figure 8A), that vector constructs with the foreign DNA insert in an E1-parallel orientation amplify better and actually out-compete E1-antiparallel-oriented transgenes. Viral DNA analysis of the mixtures at passage 3 and certainly at passage 6, showed a greater ratio of the virus carrying the transgene in the E1 parallel orientation as compared with the E1 anti-parallel version. By passage 10, the only viral species observed was the adenovector with the transgene in the E1 parallel orientation for both transgenes tested.

Adenoviral vectors in accordance with the instant invention are particularly well suited to effectuate expression of desired proteins, one example of which is an HIV protein, particularly an HIV full length gag protein. Exogenous genetic material encoding a protein of interest can exist in the form of an expression cassette. A gene expression cassette preferably comprises (a) a nucleic acid encoding a protein of interest; (b) a heterologous promoter operatively linked to the nucleic acid encoding the protein; and (c) a transcription terminator.

The transcriptional promoter is preferably recognized by an eukaryotic RNA polymerase. In a preferred embodiment, the promoter is a "strong" or "efficient" promoter. An example of a strong promoter is the immediate early human cytomegalovirus promoter (Chapman et al, 1991 *Nucl. Acids Res*19:3979-3986, which is incorporated by reference), preferably without intronic sequences. Most preferred



for use within the instant adenoviral vector is a human CMV promoter without intronic sequences, like intron A. Applicants have found that intron A, a portion of the human cytomegalovirus promoter (hCMV), constitutes a region of instability for adenoviral vectors. CMV without intron A has been found to effectuate (Examples 1-3) comparable expression capabilities *in vitro* when driving HIV gag expression and, furthermore, behaved equivalently to intron A-containing constructs in Balb/c mice *in vivo* with respect to their antibody and T-cell responses at both dosages of plasmid DNA tested (20 µg and 200 µg). Those skilled in the art will appreciate that any of a number of other known promoters, such as the strong immunoglobulin, or other eukaryotic gene promoters may also be used, including the EF1 alpha promoter, the murine CMV promoter, Rous sarcoma virus (RSV) promoter, SV40 early/late promoters and the beta-actin promoter.

In preferred embodiments, the promoter may also comprise a regulatable sequence such as the Tet operator sequence. This would be extremely useful, for example, in cases where the gene products are effecting a result other than that desired and repression is sought.

Preferred transcription termination sequences present within the gene expression cassette are the bovine growth hormone terminator/polyadenylation signal (bGHpA) and the short synthetic polyA signal (SPA) of 50 nucleotides in length, defined as follows: AATAAAAGATCTTTATTTTCATTAGATCTGTGTGTTGGT-TTTTGTGTG (SEQ ID NO:26).

The combination of the CMV promoter (devoid of the intron A region) with the BGH terminator is particularly preferred although other promoter/terminator combinations in the context of FG adenovirus may also be used.

Other embodiments incorporate a leader or signal peptide into the transgene. A preferred leader is that from the tissue-specific plasminogen activator protein, tPA. Examples include but are not limited to the various tPA-gag, tPA-pol and tPA-nef adenovirus-based vaccines disclosed throughout this specification.

In view of the improved adenovirus vectors described herein, an essential portion of the present invention are adenoviral-based HIV vaccines comprising said adenovirus backbones which may be administered to a mammalian host, preferably a human host, in either a prophylactic or therapeutic setting. The HIV vaccines of the present invention, whether administered alone or in combination regimens with other viral- or non-viral-based DNA vaccines, should elicit potent and broad cellular immune responses against HIV that will either lessen the likelihood of persistent virus infection and/or lead to the establishment of a clinically significant lowered virus load

subject to HIV infection or in combination with HAART therapy, mitigate the effects of previously established HIV infection (antiviral immunotherapy(ARI)). While any HIV antigen (e.g., gag, pol, nef, gp160, gp41, gp120, tat, rev, etc.) may be utilized in the herein described recombinant adenoviral vectors, preferred embodiments include the codon optimized p55 gag antigen (herein exemplified as MRKAd5gag), pol and nef. Sequences based on different Clades of HIV-1 are suitable for use in the instant invention, most preferred of which are Clade B and Clade C. Particularly preferred embodiments are those sequences (especially, codon-optimized sequences) based on consensus Clade B sequences. Preferred versions of the MRKAd5pol and MRKAd5nef series of adenoviral vaccines will encode modified versions of pol or nef, as discussed herein. Preferred embodiments of the MRKAd5HIV-1 vectors carrying HIV envelope genes and modifications thereof comprise the HIV codon-optimized *env* sequences of PCT International Applications PCT/US97/02294 and PCT/US97/10517, published August 28, 1997 (WO 97/31115) and December 24, 1997, respectively; both documents of which are hereby incorporated by reference.

A most preferred aspect of the instant invention is the disclosed use of the adenoviral vector described above to effectuate expression of HIV gag. Sequences for many genes of many HIV strains are publicly available in GENBANK and primary, field isolates of HIV are available from the National Institute of Allergy and Infectious Diseases (NIAID) which has contracted with Quality Biological (Gaithersburg, MD) to make these strains available. Strains are also available from the World Health Organization (WHO), Geneva Switzerland. It is preferred that the gag gene be from an HIV-1 strain (CAM-1; Myers et al, eds. "Human Retroviruses and AIDS: 1995, IIA3-IIA19, which is hereby incorporated by reference). This gene closely resembles the consensus amino acid sequence for the clade B (North American/European) sequence. Therefore, it is within the purview of the skilled artisan to choose an appropriate nucleotide sequence which encodes a specific HIV gag antigen, or immunologically relevant portion thereof. As shown in Example 25, a clade B or clade C based p55 gag antigen will potentially be useful on a global scale. As noted herein, the transgene of choice for insertion in to a DNA or MRKAd-based adenoviral vector of the present invention is a codon optimized version of p55 gag. Such a MRKAd5gag adenoviral vector is documented in Example 11 and is at least referred to herein as MRKAd5HIV-1gag. Of course, additional versions are contemplated, including but not limited to modifications such as promoter (e.g., mCMV for hCMV) and/or pA-terminations signal (SPA for bGH) switching, as well as generating MRK Ad5 backbones with or without deletion of the Ad5 E3 gene.

The present invention also relates a series of MRKAd5pol-based adenoviral vaccines which are shown herein to generate cellular immune responses subsequent to administration in mice and non-human primate studies. Several of the MRKAd5pol series are exemplified herein. One such adenoviral vector is referred to as

5 MRKAd5hCMV-inact opt pol(E3+), which comprises the MRKAd5 backbone, the hCMV promoter (no intron A), an inactivated pol transgene, and contains the Ad5 E3 gene in the adenoviral backbone. A second exemplified pre-adenovirus plasmid and concomitant virus is referred to as MRKAd5hCMV-inact opt pol(E3-), which is identical to the former adenoviral vector except that the E3 is deleted. Both

10 constructions contain a codon optimized, inactivated version of HIV-1 Pol, wherein at least the entire coding region is disclosed herein as SEQ ID NO:3 and the expressed protein is shown as SEQ ID NO:4 (see also Figure 17A-C and Table 1, which show targeted deletion for inactivated pol. This and other preferred codon optimized versions of HIV Pol as disclosed herein are essentially as described in U.S.

15 Application Serial No. 09/745,221, filed December 21, 2000 and PCT International Application PCT/US00/34724, also filed December 21, 2000, both documents which are hereby incorporated by reference. As disclosed in the above-mentioned documents, the open reading frame for these codon-optimized HIV-1 Pol-based DNA vaccines are represented by codon optimized DNA molecules encoding codon

20 optimized HIV-1 Pol (e.g. SEQ ID NO:2), codon optimized HIV-1 Pol fused to an amino terminal localized leader sequence (e.g. SEQ ID NO:6), and especially preferable, and exemplified by the MRKAd5-Pol construct in e.g., Example 19, biologically inactivated pol ("inact opt Pol"; e.g., SEQ ID NO:4) which is devoid of significant PR, RT, RNase or IN activity associated with wild type Pol. In addition, a

25 construct related to SEQ ID NO:4 is contemplated which contains a leader peptide at the amino terminal region of the IA Pol protein. A specific construct is ligated within an appropriate DNA plasmid vector containing regulatory regions operatively linked to the respective HIV-1 Pol coding region, with or without a nucleotide sequence encoding a functional leader peptide. To this end, various HIV-1 Pol constructs

30 disclosed herein relate to open reading frames for cloning to the enhanced first generation Ad vectors of the present invention (such a series of MRKAd5pol adenoviral vaccine vectors), including but not limited to wild type Pol (comprising the DNA molecule encoding WT opt Pol, as set forth in SEQ ID NO:2), tPA-opt WTPol, (comprising the DNA molecule encoding tPA Pol, as set forth in SEQ ID NO:6), inact

35 opt Pol (comprising the DNA molecule encoding IA Pol, as set forth in SEQ ID NO:4), and tPA-inact opt Pol, (comprising the DNA molecule encoding tPA-inact opt

Pol, as set forth in SEQ ID NO:8). The pol-based versions of enhanced first generation adenovirus vaccines elicit CTL and Th cellular immune responses upon administration to the host, including primates and especially humans. As noted in the above, an effect of the cellular immune-directed vaccines of the present invention should be a lower transmission rate to previously uninfected individuals and/or reduction in the levels of the viral loads within an infected individual, so as to prolong the asymptomatic phase of HIV-1 infection.

The present invention further relates to a series of MRKAd5nef-based adenoviral vaccines which, similar to HIV gag and pol antigens, generate cellular immune responses subsequent to administration in mice and non-human primate studies. The MRKAd5nef series are exemplified herein by utilizing the improved MRK adenoviral backbone in combination with modified versions of HIV nef. These exemplified MRKAd5nef vectors are as follows: (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+), which comprises the improved MRKAd5 backbone, a human CMV promoter an intact Ad5 E3 gene and a modified nef gene: (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+), which is the same as (1) above but substituting a murine CMV promoter for a human CMV promoter; and (3) MRKAd5mCMV-tpanef(LLAA) (E3+), which is the same as (2) except that the nef transgene is tpanef(LLAA). Codon optimized versions of HIV-1 Nef and HIV-1 Nef modifications are essentially as described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and PCT International Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference. Particular embodiments of codon optimized Nef and Nef modifications relate to a DNA molecule encoding HIV-1 Nef from the HIV-1 jf1 isolate wherein the codons are optimized for expression in a mammalian system such as a human. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:9, while the expressed open reading frame is disclosed herein as SEQ ID NO:10. Another embodiment of Nef-based coding regions for use in the adenoviral vectors of the present invention comprise a codon optimized DNA molecule encoding a protein containing the human plasminogen activator (tpa) leader peptide fused with the NH<sub>2</sub>-terminus of the HIV-1 Nef polypeptide. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:11, while the expressed open reading frame is disclosed herein as SEQ ID NO:12. Another modified Nef optimized coding region relates to a DNA molecule encoding optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175, herein

described as opt nef (G2A, LLAA). The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:13, while the expressed open reading frame is disclosed herein as SEQ ID NO:14. MRKAd5nef vectors (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+) and (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+) contain this transgene. An additional embodiment relates to a DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA), comprises an open reading frame which encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175, herein referred to as opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, while the expressed open reading frame is disclosed herein as SEQ ID NO:16. The MRKAd5nef vector "MRKAd5mCMV-tpanef(LLAA) (E3+)" contains this transgene.

Along with the improved MRKAd5gag adenovirus vaccine vector described herein, generation of a MRKAd5pol and MRKAd5nef adenovirus vector provide for enhanced HIV vaccine capabilities. Namely, the generation of this trio of adenoviral vaccine vectors, all shown to generate effective cellular immune responses subsequent to host administration, provide for the ability to administer these vaccine candidates not only alone, but preferably as part of a divalent (i.e., gag and nef, gag and pol, or pol and nef components) or a trivalent vaccine (i.e., gag, pol and nef components). Therefore, a preferred aspect of the present invention are vaccine formulations and associated methods of administration and concomitant generation of host cellular immune responses associated with formulating three separate series of MRKAd5-based adenoviral vector vaccines. Of course, this MRKAd5 vaccine series based on distinct HIV antigens promotes expanded opportunities for formulation of a divalent or trivalent vaccine, or possibly administration of separate formulations of one or more monovalent or divalent formulations within a reasonable window of time. It is also within the scope of the present invention to embark on combined modality regimes which include multiple but distinct components from a specific antigen. An example, but certainly not a limitation, would be separate MRKAd5pol vectors, with one vaccine vector expressing wild type Pol (SEQ ID NO:2) and another MRKAd5pol vector expressing inactivated Pol (SEQ ID NO:6). Another example might be separate MRKAd5nef vectors, with one vaccine vector expressing the tPA/LLAA version of Nef (SEQ ID NO:16) and another MRKAd5nef vector expressing the G2A,LLAA modified version of Nef (SEQ ID NO:14). Therefore, the MRKAd5 adenoviral vectors of the present invention may be used in combination

with multiple, distinct HIV antigen classes. Each HIV antigen class is subject to sequence manipulation, thus providing for a multitude of potential vaccine combinations; and such combinations are within the scope of the present invention. The utilization of such combined modalities vaccine formulation and administration  
5 increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

The present invention also relates to application of a mono-, dual-, or tri-modality administration regime of the MRKAd5gag, pol and nef adenoviral vaccine series in a prime/boost vaccination schedule. This prime/boost schedule may include  
10 any reasonable combination of the MRKAd5gag, pol and nef adenoviral vaccine series disclosed herein. In addition, a prime/boost regime may also involve other viral and/or non-viral DNA vaccines. A preferable addition to an adenoviral vaccine vector regime includes but is not limited to plasmid DNA vaccines, especially DNA plasmid vaccines that contain at least one of the codon optimized gag, pol and nef  
15 constructions, as disclosed herein.

Therefore, one aspect of this invention is the administration of the adenoviral vector containing the optimized gag gene in a prime/boost regiment in conjunction with a plasmid DNA encoding gag. To distinguish this plasmid from the adenoviral-containing shuttle plasmids used in the construction of an adenovirus vector, this  
20 plasmid will be referred to as a "vaccine plasmid" or "DNA plasmid vaccine". Preferred vaccine plasmids for use in this administration protocol are disclosed in pending U.S. patent application 09/017,981, filed February 3, 1998 and WO98/34640, published August 13, 1998, both of which are hereby incorporated by reference. Briefly, the preferred vaccine plasmid is designated V1Jns-FLgag, which expresses  
25 the same codon-optimized gag gene as the adenoviral vectors of this invention (see Figure 2 for the nucleotide sequence of the exemplified optimized codon version of full length p55 gag). The vaccine plasmid backbone, designated V1Jns contains the CMV immediate-early (IE) promoter and intron A, a bovine growth hormone-derived polyadenylation and transcription termination sequence as the gene expression  
30 regulatory elements, and a minimal pUC backbone; see Montgomery *et al.*, 1993, *DNA Cell Biol.* 12:777-783. The pUC sequence permits high levels of plasmid production in *E. coli* and has a neomycin resistance gene in place of an ampicillin resistance gene to provide selected growth in the presence of kanamycin. Alternatively, a vaccine plasmid which has the CMV promoter deleted of intron A can  
35 be used. Those of skill in the art will recognize that alternative vaccine plasmid

vectors may be easily substituted for these specific constructs, and this invention specifically envisions use of such alternative plasmid DNA vaccine vectors.

Another aspect of the present invention is a prime/boost regimen which includes a vaccine plasmid which encodes an HIV pol antigen, preferably a codon  
5 optimized form of pol and also preferably a vaccine plasmid which comprises a nucleotide sequence which encodes a Pol antigen selected from the group of Pol antigens as shown in SEQ ID NOs: 2, 4, 6 and 8. The variety of potential DNA plasmid vaccines which encode various biologically active forms of HIV-1 Pol, wherein administration, intracellular delivery and expression of the HIV-1 Pol gene of  
10 interest elicits a host CTL and Th response. The preferred synthetic DNA molecules of the present invention encode codon optimized wild type Pol (without Pro activity) and various codon optimized inactivated HIV-1 Pol proteins. The HIV-1 *pol* open reading disclosed herein are especially preferred for pharmaceutical uses, especially for human administration as delivered via a recombinant adenoviral vaccine,  
15 especially an enhanced first generation recombinant adenoviral vaccine as described herein. Several embodiments of this portion of the invention are provided in detail below, namely DNA molecules which comprise a HIV-1 pol open reading frame, whether encoding full length pol or a modification or fusion as described herein, wherein the codon usage has been optimized for expression in a mammal, especially a  
20 human. Again, these DNA sequences are positioned appropriately within a recombinant adenoviral vector, such as the exemplified recombinant adenoviral vector described herein, so as to promote expression of the respective HIV-1 Pol gene of interest, and subsequent to administration, elicit a host CTL and Th response. Again, these preferred, but in no way limiting, pol genes are as disclosed herein and  
25 essentially as described in U.S. Application Serial No. 09/745,221, filed December 21, 2000 and PCT International Application PCT/US00/34724, also filed December 21, 2000, both documents which are hereby incorporated by reference.

A third series of vaccine plasmids which are useful in a combined modality and/or prime/boost regimen are vaccine plasmids which encode an HIV nef antigen or  
30 biologically and/or immunologically relevant modification thereof. As noted elsewhere, preferred vaccine plasmids contain a codon optimized form of nef and also preferably comprise a nucleotide sequence which encodes a Nef antigen selected from the group of Nef antigens as shown in SEQ ID NOs: 10, 12, 14 and 16. These preferred nef coding regions are disclosed herein, as well as being described in U.S.  
35 Application Serial No. 09/738,782, filed December 15, 2000 and PCT International

Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference.

Therefore, the adenoviral vaccines and plasmid DNA vaccines of this invention may be administered alone, or may be part of a prime and boost administration regimen. A mixed modality priming and booster inoculation scheme will result in an enhanced immune response, particularly if pre-existing anti-vector immune responses are present. This one aspect of this invention is a method of priming a subject with the plasmid vaccine by administering the plasmid vaccine at least one time, allowing a predetermined length of time to pass, and then boosting by administering the adenoviral vaccine. Multiple primings typically, 1-4, are usually employed, although more may be used. The length of time between priming and boost may typically vary from about four months to a year, but other time frames may be used. In experiments with rhesus monkeys, the animals were primed four times with plasmid vaccines, then were boosted 4 months later with the adenoviral vaccine. Their cellular immune response was notably higher than that of animals which had only received adenoviral vaccine. The use of a priming regimen may be particularly preferred in situations where a person has a pre-existing anti-adenovirus immune response.

Furthermore and in the alternative, multiple HIV-1 viral antigens, such as the MRKAd5 adenoviral vaccines disclosed herein, may be ligated into a proper shuttle plasmid for generation of a pre-adenoviral plasmid comprising multiple open reading frames. For example a trivalent vector may comprise a gag-pol-nef fusion, in either a E3(-) or E3(+) background, preferably a E3 deleted backbone, or possibly a "2+1" divalent vaccine, such as a gag-pol fusion (i.e., codon optimized p55 gag and inactivated optimized pol; Example 29 and Table 25) within the same MRKAd5 backbone, with each open reading frame being operatively linked to a distinct promoter and transcription termination sequence. Alternatively, the two open reading frames may be operatively linked to a single promoter, with the open reading frames operatively linked by an internal ribosome entry sequence (IRES), as disclosed in International Publication No. WO 95/24485, which is hereby incorporated by reference. Figure 9 shows that the use of multiple promoters and termination sequences provide for similar growth properties, while Figure 28 shows that these MRKAd5gag-based vectors are also stable at least through passage 21. In the absence of the use of IRES-based technology, it is preferred that a distinct promoter be used to support each respective open reading frame, so as to best preserve vector stability. As examples, and certainly not as limitations, potential multiple transgene vaccines may



include a three transgene vector such as hCMV-gagpol-bGHpA + mCMV-nef-SPA in an E3 deleted backbone or hCMV-gagpol-bGHpA + mCMV-nef-SPA(E3+).

Potential "2+1" divalent vaccines of the present invention might be a hCMV-gag-bGHpA + mCMV-nef-SPA in an E3+ backbone (vector #1) in combination with  
5 hCMV-pol-bGHpA in an E3+ backbone (vector #2), with all transgenes in the E1 parallel orientation. Fusion constructs other than the gag-pol fusion described above are also suitable for use in various divalent vaccine strategies and can be composed of any two HIV antigens fused to one another (*e.g.*, nef-pol and gag-nef). These  
10 adenoviral compositions are, as above, preferably delivered along with an adenoviral composition comprising an additional HIV antigen in order to diversify the immune response generated upon administration. Therefore, a multivalent vaccine delivered in a single, or possible second, adenoviral vector is certainly contemplated as part of the present invention. Again, this mode of administration is another example of  
15 whereby an efficacious adenovirus-based HIV-1 vaccine may be administered via a combined modality regime. It is important to note, however, that in terms of deciding on an insert for the disclosed adenoviral vectors, due consideration must be dedicated to the effective packaging limitations of the adenovirus vehicle. Adenovirus has been shown to exhibit an upper cloning capacity limit of approximately 105% of the  
20 wildtype Ad5 sequence.

Regardless of the gene chosen for expression, it is preferred that the sequence be "optimized" for expression in a human cellular environment. A "triplet" codon of four possible nucleotide bases can exist in 64 variant forms. That these forms provide the message for only 20 different amino acids (as well as transcription initiation and termination) means that some amino acids can be coded for by more than one codon.  
25 Indeed, some amino acids have as many as six "redundant", alternative codons while some others have a single, required codon. For reasons not completely understood, alternative codons are not at all uniformly present in the endogenous DNA of differing types of cells and there appears to exist variable natural hierarchy or "preference" for certain codons in certain types of cells. As one example, the amino  
30 acid leucine is specified by any of six DNA codons including CTA, CTC, CTG, CTT, TTA, and TTG (which correspond, respectively, to the mRNA codons, CUA, CUC, CUG, CUU, UUA and UUG). Exhaustive analysis of genome codon frequencies for microorganisms has revealed endogenous DNA of *E. coli* most commonly contains the CTG leucine-specifying codon, while the DNA of yeasts and slime molds most  
35 commonly includes a TTA leucine-specifying codon. In view of this hierarchy, it is generally held that the likelihood of obtaining high levels of expression of a leucine-

rich polypeptide by an *E. coli* host will depend to some extent on the frequency of codon use. For example, a gene rich in TTA codons will in all probability be poorly expressed in *E. coli*, whereas a CTG rich gene will probably highly express the polypeptide. Similarly, when yeast cells are the projected transformation host cells  
5 for expression of a leucine-rich polypeptide, a preferred codon for use in an inserted DNA would be TTA.

The implications of codon preference phenomena on recombinant DNA techniques are manifest, and the phenomenon may serve to explain many prior failures to achieve high expression levels of exogenous genes in successfully  
10 transformed host organisms--a less "preferred" codon may be repeatedly present in the inserted gene and the host cell machinery for expression may not operate as efficiently. This phenomenon suggests that synthetic genes which have been designed to include a projected host cell's preferred codons provide a preferred form of foreign genetic material for practice of recombinant DNA techniques. Thus, one aspect of  
15 this invention is an adenovirus vector or adenovirus vector in some combination with a vaccine plasmid where both specifically include a gene which is codon optimized for expression in a human cellular environment. As noted herein, a preferred gene for use in the instant invention is a codon-optimized HIV gene and, particularly, HIV gag, pol or nef.

20 Adenoviral vectors in accordance with the instant invention can be constructed using known techniques, such as those reviewed in Hitt et al, 1997 "Human Adenovirus Vectors for Gene Transfer into Mammalian Cells" *Advances in Pharmacology* 40:137-206, which is hereby incorporated by reference.

In constructing the adenoviral vectors of this invention, it is often convenient  
25 to insert them into a plasmid or shuttle vector. These techniques are known and described in Hitt et al., *supra*. This invention specifically includes both the adenovirus and the adenovirus when inserted into a shuttle plasmid.

Preferred shuttle vectors contain an adenoviral portion and a plasmid portion. The adenoviral portion is essentially the same as the adenovirus vector discussed  
30 *supra*, containing adenoviral sequences (with non-functional or deleted E1 and E3 regions) and the gene expression cassette, flanked by convenient restriction sites. The plasmid portion of the shuttle vector often contains an antibiotic resistance marker under transcriptional control of a prokaryotic promoter so that expression of the antibiotic does not occur in eukaryotic cells. Ampicillin resistance genes, neomycin  
35 resistance genes and other pharmaceutically acceptable antibiotic resistance markers may be used. To aid in the high level production of the polynucleotide by

fermentation in prokaryotic organisms, it is advantageous for the shuttle vector to contain a prokaryotic origin of replication and be of high copy number. A number of commercially available prokaryotic cloning vectors provide these benefits. It is desirable to remove non-essential DNA sequences. It is also desirable that the vectors not be able to replicate in eukaryotic cells. This minimizes the risk of integration of polynucleotide vaccine sequences into the recipients' genome. Tissue-specific promoters or enhancers may be used whenever it is desirable to limit expression of the polynucleotide to a particular tissue type.

In one embodiment of this invention, the pre-plasmids (e.g., pMRKAd5pol, pMRKAd5nef and pMRKAd5gag) were generated by homologous recombination using the MRKHVE3 (and MRKHVO for the E3- version) backbones and the appropriate shuttle vector, as shown for pMRKAd5pol in Figure 22 and for pMRKAd5nef in Figure 23. The plasmid in linear form is capable of replication after entering the PER.C6<sup>®</sup> cells and virus is produced. The infected cells and media were harvested after viral replication was complete.

Viral vectors can be propagated in various E1 complementing cell lines, including the known cell lines 293 and PER.C6<sup>®</sup>. Both these cell lines express the adenoviral E1 gene product. PER.C6<sup>®</sup> is described in WO 97/00326 (published January 3, 1997) and issued U.S. Patent No. 6,033,908, both of which are hereby incorporated by reference. It is a primary human retinoblast cell line transduced with an E1 gene segment that complements the production of replication deficient (FG) adenovirus, but is designed to prevent generation of replication competent adenovirus by homologous recombination. Cells of particular interest have been stably transformed with a transgene that encodes the AD5E1A and E1B gene, like PER.C6<sup>®</sup>, from 459 bp to 3510 bp inclusive. 293 cells are described in Graham et al., 1977 *J. Gen. Virol* 36:59-72, which is hereby incorporated by reference. As stated above, consideration must be given to the adenoviral sequences present in the complementing cell line used. It is important that the sequences not overlap with that present in the vector if the possibility of recombination is to be minimized.

It has been found that vectors generated in accordance with the above description are more effective in inducing an immune response and, thus, constitute very promising vaccine candidates. More particularly, it has been found that first generation adenoviral vectors in accordance with the above description carrying a codon-optimized HIV gag gene, regulated with a strong heterologous promoter can be used as human anti-HIV vaccines, and are capable of inducing immune responses.

Standard techniques of molecular biology for preparing and purifying DNA constructs enable the preparation of the DNA immunogens of this invention.

A vaccine composition comprising an adenoviral vector in accordance with the instant invention may contain physiologically acceptable components, such as  
5 buffer, normal saline or phosphate buffered saline, sucrose, other salts and polysorbate. One preferred formulation has: 2.5-10 mM TRIS buffer, preferably about 5 mM TRIS buffer; 25-100 mM NaCl, preferably about 75 mM NaCl; 2.5-10% sucrose, preferably about 5% sucrose; 0.01 -2 mM  $MgCl_2$ ; and 0.001%-0.01% polysorbate 80 (plant derived). The pH should range from about 7.0-9.0, preferably  
10 about 8.0. One skilled in the art will appreciate that other conventional vaccine excipients may also be used it make the formulation. The preferred formulation contains 5mM TRIS, 75 mM NaCl, 5% sucrose, 1mM  $MgCl_2$ , 0.005% polysorbate 80 at pH 8.0 This has a pH and divalent cation composition which is near the optimum for Ad5 stability and minimizes the potential for adsorption of virus to a glass surface.  
15 It does not cause tissue irritation upon intramuscular injection. It is preferably frozen until use.

The amount of adenoviral particles in the vaccine composition to be introduced into a vaccine recipient will depend on the strength of the transcriptional and translational promoters used and on the immunogenicity of the expressed gene  
20 product. In general, an immunologically or prophylactically effective dose of  $1 \times 10^7$  to  $1 \times 10^{12}$  particles and preferably about  $1 \times 10^{10}$  to  $1 \times 10^{11}$  particles is administered directly into muscle tissue. Subcutaneous injection, intradermal introduction, impression through the skin, and other modes of administration such as intraperitoneal, intravenous, or inhalation delivery are also contemplated. It is also  
25 contemplated that booster vaccinations are to be provided. Following vaccination with HIV adenoviral vector, boosting with a subsequent HIV adenoviral vector and/or plasmid may be desirable. Parenteral administration, such as intravenous, intramuscular, subcutaneous or other means of administration of interleukin-12 protein, concurrently with or subsequent to parenteral introduction of the vaccine  
30 compositions of this invention is also advantageous.

The adenoviral vector and/or vaccine plasmids of this invention polynucleotide may be unassociated with any proteins, adjuvants or other agents which impact on the recipients' immune system. In this case, it is desirable for the vector to be in a physiologically acceptable solution, such as, but not limited to, sterile  
35 saline or sterile buffered saline. Alternatively, the vector may be associated with an adjuvant known in the art to boost immune responses (i.e., a "biologically effective"

adjuvant), such as a protein or other carrier. Vaccine plasmids of this invention may, for instance, be delivered in saline (e.g., PBS) with or without an adjuvant. Preferred adjuvants are Alum or CRL1005 Block Copolymer. Agents which assist in the cellular uptake of DNA, such as, but not limited to, calcium ions, may also be used to advantage. These agents are generally referred to herein as transfection facilitating reagents and pharmaceutically acceptable carriers. Techniques for coating microprojectiles coated with polynucleotide are known in the art and are also useful in connection with this invention.

This invention also includes a prime and boost regimen wherein a first adenoviral vector is administered, then a booster dose is given. The booster dose may be repeated at selected time intervals. Alternatively, a preferred inoculation scheme comprises priming with a first adenovirus serotype and then boosting with a second adenovirus serotype. More preferably, the inoculation scheme comprises priming with a first adenovirus serotype and then boosting with a second adenovirus serotype, wherein the first and second adenovirus serotypes are classified within separate subgroups of adenoviruses. The above prime/boost schemes are particularly preferred in those situations where a preexisting immunity is identified to the adenoviral vector of choice. In this type of scheme, the individual or population of individuals is primed with an adenovirus of a serotype other than that to which the preexisting immunity is identified. This enables the first adenovirus to effectuate sufficient expression of the transgene while evading existing immunity to the second adenovirus (the boosting adenovirus) and, further, allows for the subsequent delivery of the transgene via the boosting adenovirus to be more effective. Adenovirus serotype 5 is one example of a virus to which such a scheme might be desirable. In accordance with this invention, therefore, one might decide to prime with a non-group C adenovirus (e.g., Ad12, a group A adenovirus, Ad24, a group D adenovirus, or Ad35, a group B adenovirus) to evade anti-Ad5 immunity and then boost with Ad5, a group C adenovirus. Another preferred embodiment involves administration of a different adenovirus (including non-human adenovirus) vaccine followed by administration of the adenoviral vaccines disclosed. In the alternative, a viral antigen of interest can be first delivered via a viral vaccine other than an adenovirus-based vaccine, and then followed with the adenoviral vaccine disclosed. Alternative viral vaccines include but are not limited to pox virus and venezuelan equine encephalitis virus.

A large body of human and animal data supports the importance of cellular immune responses, especially CTL in controlling (or eliminating) HIV infection. In humans, very high levels of CTL develop following primary infection and correlate

with the control of viremia. Several small groups of individuals have been described who are repeatedly exposed to HIV by remain uninfected; CTL has been noted in several of these cohorts. In the SIV model of HIV infection, CTL similarly develops following primary infection, and it has been demonstrated that addition of anti-CD8 monoclonal antibody abrogated this control of infection and leads to disease progression. This invention uses adenoviral vaccines alone or in combination with plasmid vaccines to induce CTL.

The following non-limiting Examples are presented to better illustrate the invention.

#### EXAMPLE 1

##### Removal of the Intron A Portion of the hCMV Promoter

GMP grade pVJnsHIVgag was used as the starting material to amplify the hCMV promoter. PVIJnsHIVgag is a plasmid comprising the CMV immediate-early (IE) promoter and intron A, a full-length codon-optimized HIV gag gene, a bovine growth hormone-derived polyadenylation and transcriptional termination sequence, and a minimal pUC backbone; see Montgomery *et al.*, *supra* for a description of the plasmid backbone. The amplification was performed with primers suitably positioned to flank the hCMV promoter. A 5' primer was placed upstream of the *MscI* site of the hCMV promoter and a 3' primer (designed to contain the *BglIII* recognition sequence) was placed 3' of the hCMV promoter. The resulting PCR product (using high fidelity *Taq* polymerase) which encompassed the entire hCMV promoter (minus intron A) was cloned into TOPO PCR blunt vector and then removed by double digestion with *MscI* and *BglIII*. This fragment was then cloned back into the original GMP grade pV1JnsHIVgag plasmid from which the original promoter, intron A, and the gag gene were removed following *MscI* and *BglIII* digestion. This ligation reaction resulted in the construction of a hCMV promoter (minus intron A) + bGHpA expression cassette within the original pV1JnsHIVgag vector backbone. This vector is designated pVJnsCMV(no intron).

The FLgag gene was excised from pV1JnsHIVgag using *BglIII* digestion and the 1,526 bp gene was gel purified and cloned into pV1JnsCMV(no intron) at the *BglIII* site. Colonies were screened using *SmaI* restriction enzymes to identify clones that carried the FLgag gene in the correct orientation. This plasmid, designated pV1JnsCMV(no intron)-FLgag-bGHpA, was fully sequenced to confirm sequence integrity.

Two additional transgenes were also constructed. The plasmid, pV1JnsCMV(no intron)-FLgag-SPA, is identical to pV1JnsCMV(no intron)-FLgag-bGHpA except that the bovine growth hormone polyadenylation signal has been replaced with a short synthetic polyA signal (SPA) of 50 nucleotides in length. The sequence of the SPA is as shown, with the essential components (poly(A) site, (GT)<sub>n</sub>, and (T)<sub>n</sub>; respectively) underlined:

AATAAAAGATCTTTATTTTCATTAGATCTGTGTG TTGGTTTTTTGTGTG  
(SEQ ID NO:18).

The plasmid, pV1Jns-mCMV-FLgag-bGHpA, is identical to the pV1JnsCMV(no intron)-FLgag-bGHpA except that the hCMV promoter has been removed and replaced with the murine CMV (mCMV) promoter.

Figure 3 diagrammatically shows the new transgene constructs in comparison with the original transgene.

## EXAMPLE 2

### Gag Expression Assay for Modified Gag Transgenes

Gag Elisa was performed on culture supernatants obtained from transient tissue culture transfection experiments in which the two new hCMV-containing plasmid constructs, pV1JnsCMV(no intron)-FLgag-bGHpA and pV1JnsCMV(no intron)-FLgag-SPA, both devoid of intron A, were compared to pV1JnsHIVgag which, as noted above possesses the intron A as part of the hCMV promoter. Table 2 below shows the *in vitro* gag expression data of the new gag plasmids compared with the GMP grade original plasmid. The results displayed in Table 2 show that both of the new hCMV gag plasmid constructs have expression capacities comparable to the original plasmid construct which contains the intron A portion of the hCMV promoter.

Table 2: *In vitro* DNA transfection of original and new plasmid HIV-1 gag constructs.

Plasmid	$\mu\text{g gag}/10^6 \text{ COS cells}/5\mu\text{g DNA}/48 \text{ hr}$
HIVFL-gagPR9901 <sup>a</sup>	10.8
PV1Jns-hCMV-FLgag-bGHpA <sup>b</sup>	16.6
pV1Jns-hCMV-FLgag-SPA <sup>bc</sup>	12.0

<sup>a</sup> GMP grade pV1Jns-hCMVintronA-FLgag-bGHpA.

5 <sup>b</sup> New plasmid constructions that have the intron A portion removed from the hCMV promoter.

<sup>c</sup> In this construct the bGH terminator has been replaced with the short synthetic polyadenylation signal (SPA)

### 10 EXAMPLE 3

#### Rodent (Balb/c) Study for Modified gag Transgenes

A rodent study was performed on the two new plasmid constructs described above – pV1JnsCMV(no intron)-FLgag-bGHpA and pV1JnsCMV(no intron)-FLgag-SPA - in order to compare them with the construct described above  
 15 possessing the intron A portion of the CMV promoter, pV1JnsHIVgag. Gag antibody and Elispot responses (described in PCT International Application No. PCT/US00/18332 (WO 01/02607) filed July 3, 2000, claiming priority to U.S. Provisional Application Serial No. 60/142,631, filed July 6, 1999 and U.S. Application Serial No. 60/148,981, filed August 13, 1999, all three applications which  
 20 are hereby incorporated by reference) were measured. The results displayed in Table 3 below, show that the new plasmid constructs behaved equivalently to the original construct in Balb/c mice with respect to their antibody and T-cell responses at both dosages of plasmid DNA tested, 20  $\mu\text{g}$  and 200  $\mu\text{g}$ .



## EXAMPLE 4

Table 3: HIV191: Immunogenicity of V1Jns-gag under different promoter and termination control elements.

DNA <sup>a</sup> Promoter/terminator	Dose, ug <sup>b</sup>	Anti-p24 Titers (3 Wk PD1) <sup>c</sup>			SFC/10 <sup>6</sup> Cells (4 Wk PD1) <sup>d</sup>		
		GMT	+SE	-SE	Media	gag197-205	p24
HIVFL-gagPR9901 (GMP grade)	200	12800	4652	3412	2(2)	129(19)	30(11)
	20	5572	1574	1227	0	56(9)	25(6)
pV1Jns-hCMV- FL-gag-bGHpA	200	11143	2831	2257	0	98(5)	12(6)
	20	7352	2808	2032	0	73(9)	11(6)
pV1Jns-hCMV- FL-gag-SPA	200	16890	5815	4326	1(1)	94(4)	26(7)
	20	5971	5361	2825	0	85(17)	38(10)
Naïve	0	123	50	36	0	0	0

<sup>a</sup>in PBS<sup>b</sup>i.m. Injections into both quads, 50 µL per quad<sup>c</sup>n=10; GMT, geometric mean titer; SE, standard. error<sup>d</sup>n=5, pooled spleens; mean of triplicate wells and standard. deviation. in parentheses;

## Construction of the Modified Shuttle Vector -"MRKpdeIE1 Shuttle"

- The modifications to the original Ad5 shuttle vector (pdeIE1sp1A; a vector comprising Ad5 sequences from basepairs 1-341 and 3524-5798, with a multiple cloning region between nucleotides 341 and 3524 of Ad5, included the following three manipulations carried out in sequential cloning steps as follows:
- (1) The left ITR region was extended to include the *Pac*1 site at the junction between the vector backbone and the adenovirus left ITR sequences. This allow for easier manipulations using the bacterial homologous recombination system.
  - (2) The packaging region was extended to include sequences of the wild-type (WT) adenovirus from 342 bp to 450 bp inclusive.
  - (3) The area downstream of pIX was extended 13 nucleotides (i.e., nucleotides 3511-3523 inclusive).

These modifications (Figure 4) effectively reduced the size of the E1 deletion without overlapping with any part of the E1A/E1B gene present in the transformed PER.C6<sup>®</sup> cell line. All manipulations were performed by modifying the Ad shuttle vector pdeIE1sp1A.

Once the modifications were made to the shuttle vector, the changes were incorporated into the original Ad5 adenovector backbones (pAdHVO and pAdHVE3) by bacterial homologous recombination using *E. coli* BJ5183 chemically competent cells.

## EXAMPLE 5

Construction of Modified Adenovector Backbones (E3+ and E3-)

The original adenovectors pAdHVO (comprising all Ad5 sequences except those nucleotides encompassing the E1 and E3 regions ) and pAdHVE3 (comprising all Ad5 sequences except those nucleotides encompassing the E1 region), were each reconstructed so that they contained the modifications to the E1 region. This was accomplished by digesting the newly modified shuttle vector (MRKpdeIE1 shuttle) with *PacI* and *BstZ1101* and isolating the 2,734 bp fragment which corresponds to the adenovirus sequence. This fragment was co-transformed with DNA from either *ClaI* linearized pAdHVO (E3- adenovector) or *ClaI* linearized pAdHVE3 (E3+adenovector) into *E. coli* BJ5183 competent cells. At least two colonies from each transformation were selected and grown in Terrific™ broth for 6-8 hours until turbidity was reached. DNA was extracted from each cell pellet and then transformed into *E. coli* XL1 competent cells. One colony from each transformation was selected and grown for plasmid DNA purification. The plasmid was analyzed by restriction digestions to identify correct clones. The modified adenovectors were designated MRKpAdHVO (E3- plasmid) and MRKpAdHVE3 (E3+ plasmid). Virus from these new adenovectors (MRKHVO and MRKHVE3, respectively) as well as the old version of the adenovectors were generated in the PER.C6® cell lines to accommodate the following series of viral competition experiments. In addition, the multiple cloning site of the original shuttle vector contained *ClaI*, *BamHI*, *XhoI*, *EcoRV*, *HindIII*, *SalI*, and *BglII* sites. This MCS was replaced with a new MCS containing *NotI*, *ClaI*, *EcoRV* and *AscI* sites. This new MCS has been transferred to the MRKpAdHVO and MRKpAdHVE3 pre-plasmids along with the modification made to the packaging region and pIX gene.

## EXAMPLE 6

Analysis of the Effect of the Packaging Signal Extension

To study the effects of the modifications made to the E1 deletion region, the viruses obtained from the original backbone (pAdHVE3) and the new backbone (MRKpAdHVE3) were mixed together in equal MOI ratios (1:1 and 5:5) and passaged through several rounds; see Figure 5, Expt.#1. Both of the viruses in the experiment contained the E3 gene intact and did not contain a transgene. The only difference between the two viruses was within the region of the E1 deletion. Following the coinfection of the viruses at P1 (passage 1), the mixtures were propagated through an additional 4 passages at which time the cells were harvested

and the virus extracted and purified by CsCl banding. The viral DNA was extracted and digested with *HindIII* and the digestion products were then radioactively labeled. For the controls, the respective pre-plasmids (pAdHVE3 ("OLD E3+"); MRKpAdHVE3 ("NEW E3+")) were also digested with *HindIII* (and *PacI* to remove the vector backbone) and subsequently labeled with [<sup>33</sup>P]dATP. The radioactively labeled digestion products were subjected to gel electrophoresis and the gel was dried down onto Whatman paper before being exposed to autoradiographic film. Figure 6 clearly shows that the new adenovirus which has the addition made to the packaging signal region has a growth advantage compared with the original adenovirus. In the experiments performed (at either ratio tested), only the digestion bands pertaining to the newly modified virus were present. The diagnostic band of size 3,206 (from the new virus) was clearly present. However, there was no evidence of the diagnostic band of size 2,737 bp expected from the original virus.

#### EXAMPLE 7

##### Analysis of the Effect of the E3 Gene

The second set of the virus competition study involved mixing equal MOI ratio (1:1) of the newly modified viruses, that obtained from MRKpAdHVO and MRKpAdHVE3 (Figure 5, Expt. #2). In this set, both viruses had the new modifications made to the E1 deletion. The first virus (that from MRKpAdHVO) does not contain an E3 gene. The second virus (that from MRKpAdHVE3) does contain the E3 gene. Neither of the viruses contain a transgene. Following co-infection of the viruses, the mixtures were propagated through an additional 4 passages at which time the cells were harvested and the total virus extracted and purified by CsCl banding. The viral DNA was extracted and digested with *HindIII* and the digestion products were then radioactively labeled. For the controls, the respective pre-plasmids MRKpAdHVO ("NEW E3-"); MRKpAdHVE3 ("NEW E3+") were also digested with *HindIII* (and *PacI* to remove the vector backbone) and then labeled with [<sup>33</sup>P]dATP. The radioactively labeled digestion products were subjected to gel electrophoresis and the gel was dried down onto Whatman paper before being exposed to autoradiographic film. Figure 6 shows the results of the viral DNA analysis of the E3+ virus and E3- virus mixing experiment. The diagnostic band corresponding to the E3+ virus (5,665 bp) was present in greater amount compared with the diagnostic band of 3,010 bp corresponding to the E3- virus. This indicates that the virus that contains the E3 gene is able to amplify more rapidly

compared with the virus that does not contain an E3 gene. This increased amplification capacity has been confirmed by growth studies; see Table 4 below.

#### EXAMPLE 8

##### Construction of the new shuttle vector containing modified gag transgene – “MRKpdeIE1-CMV(no intron)-FLgag-bGHpA”

The modified plasmid pV1JnsCMV(no intron)-FLgag-bGHpA was digested with *MscI* overnight and then digested with *SfiI* for 2 hours at 50°C. The DNA was then treated with Mungbean nuclease for 30 mins at 30°C. The DNA mixture was desalted using the Qiaex II kit and then Klenow treated for 30 mins at 37°C to fully blunt the ends of the transgene fragment. The 2,559 bp transgene fragment was then gel purified. The modified shuttle vector (MRKpdeIE1 shuttle) was linearized by digestion with *EcoRV*, treated with calf intestinal phosphatase and the resulting 6,479 bp fragment was then gel purified. The two purified fragments were then ligated together and several dozen clones were screened to check for insertion of the transgene within the shuttle vector. Diagnostic restriction digestion was performed to identify those clones carrying the transgene in the E1 parallel and E1 anti-parallel orientation. This strategy was followed to clone in the other gag transgenes in the MRKpdeIE1 shuttle vector.

#### EXAMPLE 9

##### Construction of the MRK FG Adenovectors

The shuttle vector containing the HIV-1 gag transgene in the E1 parallel orientation, MRKpdeIE1-CMV(no intron)-FLgag-bGHpA, was digested with *PacI*. The reaction mixture was digested with *BsfZ171*. The 5,291 bp fragment was purified by gel extraction. The MRKpAdHVE3 plasmid was digested with *ClaI* overnight at 37°C and gel purified. About 100 ng of the 5,290 bp shuttle +transgene fragment and ~100 ng of linearized MRKpAdHVE3 DNA were co-transformed into *E. coli* BJ5183 chemically competent cells. Several clones were selected and grown in 2 ml Terrific™ broth for 6-8 hours, until turbidity was reached. The total DNA from the cell pellet was purified using Qiagen alkaline lysis and phenol chloroform method. The DNA was precipitated with isopropanol and resuspended in 20 µl dH<sub>2</sub>O. A 2 µl aliquot of this DNA was transformed into *E. coli* XL-1 competent cells. A single colony from each separate transformation was selected and grown overnight in 3 ml LB +100 µg/ml ampicillin. The DNA was isolated using Qiagen columns. A positive clone was identified by digestion with the restriction enzyme *BsrEII* which cleaves

within the gag gene as well as the plasmid backbone. The pre-plasmid clone is designated MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA and is 37,498 bp in size. This strategy was followed to generate E3- and E3+ versions of each of the other gag transgene constructions in both E1 parallel and E1 anti-parallel versions. Figures 7A, 7B and 7C show the various combinations of adenovectors constructed.

## EXAMPLE 10

### Plasmid Competition Studies

A series of plasmid competition studies was carried out. Briefly, the screening of the various combinations of new constructs was performed by mixing equal amounts of each of two competing plasmids. In the experiment shown in Figure 8A, plasmids containing the same transgene but in different orientations were mixed together to create a "competition" between the two plasmids. The aim was to look at the effects of transgene orientation. In the experiment shown in Figure 8B, plasmids containing different polyadenylation signals (but in the same orientation) were mixed together in equal amounts. The aim was to assess effects of polyA signals. Following the initial transfection, the virus was passaged through ten rounds and the viral DNA analyzed by radioactive restriction analysis.

Analysis of the viral species from the plasmid mixing experiment (Figure 8A) showed that adenovectors which had the transgene inserted in the E1 parallel orientation amplified better and were able to out-compete the adenovirus which had the transgene inserted in the E1 anti-parallel orientation. Viral DNA analysis of the mixtures at passage 3 and certainly at passage 6, showed a greater ratio of the virus carrying the transgene in the E1 parallel orientation compared with the E1 antiparallel version. By passage 10, the only viral species observed was the adenovector with the transgene in the E1 parallel orientation for both transgenes tested (hCMV(no intron)-FLgag-bGHpA and hCMV(no intron)-FLgag-SPA).

Analysis of the viral species from the plasmid mixing experiment #2 (Figure 8B) at passages 3 and 6 showed that the polyadenylation signals tested (bGHpA and SPA) did not have an effect on the growth of the virus. Even at passage 10 the two viral species in the mixture were still present in equal amounts.

## EXAMPLE 11

Virus generation of an enhanced adenoviral construct – “MRK Ad5 HIV-1 gag”

The results obtained from the competition study allowed us to make the following conclusions: (1) The packaging signal extension is beneficial; (2) Presence  
5 of E3 does enhance viral growth; (3) E1 parallel orientation is recommended; and (4) PolyA signals have no effect on the growth of the adenovirus.

MRK Ad5 HIV-1 gag exhibited the most desirable results. This construct contains the hCMV(no intron)-FLgag-bGHpA transgene inserted into the new E3+ adenovector backbone, MRKpAdHVE3, in the E1 parallel orientation. We have  
10 designated this adenovector MRK Ad5 HIV-1 gag. This construct was prepared as outlined below:

The pre-plasmid MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA was digested with *Pac1* to release the vector backbone and 3.3 µg was transfected by calcium phosphate method (Amersham Pharmacia Biotech.) in a 6 cm dish containing  
15 PER.C6<sup>®</sup> cells at ~60% confluence. Once CPE was reached (7-10 days), the culture was freeze/thawed three times and the cell debris pelleted. 1 ml of this cell lysate was used to infect into a 6 cm dish containing PER.C6<sup>®</sup> cells at 80-90% confluence. Once CPE was reached, the culture was freeze/thawed three times and the cell debris pelleted. The cell lysate was then used to infect a 15 cm dish containing PER.C6<sup>®</sup>  
20 cells at 80-90% confluence. This infection procedure was continued and expanded at passage 6. The virus was then extracted from the cell pellet by CsCl method. Two bandings were performed (3-gradient CsCl followed by a continuous CsCl gradient). Following the second banding, the virus was dialyzed in A105 buffer. Viral DNA was extracted using pronase treatment followed by phenol chloroform. The viral  
25 DNA was then digested with *HindIII* and radioactively labeled with [<sup>33</sup>P]dATP. Following gel electrophoresis to separate the digestion products the gel was dried down on Whatman paper and then subjected to autoradiography. The digestion products were compared with the digestion products from the pre-plasmid (that had been digested with *Pac1/HindIII* prior to labeling). The expected sizes were  
30 observed, indicating that the virus had been successfully rescued. This strategy was used to rescue virus from each of the various adenovector plasmid constructs prepared.

## EXAMPLE 12

Stability Analyses

To determine whether the various adenovector constructs (e.g., MRK Ad5 HIV-1 gag) show genetic stability, the viruses were each passaged continually. The viral DNA was analyzed at passages 3, 6 and 10. Each virus maintained its correct genetic structure. In addition, the stability of the MRK Ad5 HIV-1 gag was analyzed under propagation conditions similar to that performed in large scale production. For this analysis, the transfections of MRK Ad5 HIV-1 gag as well as three other adenoviral vectors were repeated and the virus was purified at P3. The three other adenovectors were as follows: (1) that comprising hCMV(no intron)-Flgag with a bGHpA terminator in an E3- adenovector backbone; (2) that comprising hCMV(no intron)-Flgag with a SPA termination signal in an E3+ adenovector backbone, and that comprising a mCMV-Flgag with a bGHpA terminator in an E3+ adenovector backbone. All of the vectors have the transgene inserted in the E1 parallel orientation. Viral DNA was analyzed by radioactive restriction analysis to confirm that it was correct before being delivered to fermentation cell culture for continued passaging in serum-free media. At P5 each of the four viruses were purified and the viral DNA extracted for analysis by the restriction digestion and radiolabeling procedure. This virus has subsequently been used in a series of studies (*in vitro* gag expression in COS cells, rodent study and rhesus monkey study) as will be described below. The viruses from P5 are shown in Figure 9.

The passaging under serum-free conditions was continued for the MRKHVE3 (transgene-less, obtained from MRKpAdHVE3 pre-plasmid) and the MRKAd5HIV-1gag (obtained from MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA pre-plasmid) viruses. Figure 10 shows viral DNA analysis by radioactive restriction digestion at passage 11 for MRKHVE3, MRKAd5HIV-1gagE3-, and passage 11 and 12 for MRKAd5HIV-1gag. Aside from the first lane which is the DNA marker lane, the next three lanes are virus from the pre-plasmid controls (controls based on the original virus) - MRKpAdHVE3 (also referred to as "pMRKHVE3"), MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA, and pMRKAd5gag(E3-), respectively. As seen in Figure 10, each of the viral DNA samples show the expected bands with no extraneous bands showing. This signifies that there are no major variant adenovirus species present that can be detected by autoradiography.

Figure 11 shows the results of viral competition study between MRKHVE3 and MRKAd5HIV-1gag. These viruses were mixed together at equal MOI (140 viral

particles each; 280 vp total) at passage 6 and continued to be passaged until P11.

Aside from the first lane which is the DNA marker lane, the next two lanes are the pre-plasmid controls obtained from MRKpAdHVE3 and MRKpAdHVE3+CMV(no intron)-FLgag-bGHPA. The next two lanes are the viral DNA from the starting viral material at passage six. The last two lanes are the competition studies performed in duplicate. The data in Figure 11 shows the effect the gag transgene in culture.

5 Growth of a MRKAd5gag virus was compared with growth of a "transgene-less" MRKHVE3. These two viruses were infected at the same MOI (i.e. 140 vp each) at passage 6 and then passaged through to passage 11 and the viral pool was analyzed by radioactive restriction analysis. The data shows that one virus did not out compete the other. Therefore, the gag transgene did not show obvious signs of toxicity to the adenovirus.

15 Analysis by *HindIII* digestion shows that each virus specie is present in approximately equal amounts. As above, there does not appear to be signs of any extraneous bands. Figure 12 shows higher passage numbers for MRKAd5HIV-1gag grown under serum-containing conditions. The genome integrity again has been maintained and there is no evidence of rearrangements, even at the highest passage level (P21).

20 Each of the four vectors shown in Figure 9 were analyzed for amplification capacity. Table 4 below shows the QPA analysis used in the estimation of viral amplification ratios at P4. The determination of the amplification ratio for the original HIV-1 gag construct is based on the clinical lot at P12. It has been shown that amplification rates increases with higher passage number for the original virus. The reason for this observation is due to the emergence of variants which exhibit increased growth rates compared to the intact adenovector. With continued passaging of the original Ad gag vector, the level of variants increases and hence amplification rates increase also.

25 The MRK Ad5 HIV-1 gag virus has also been continually passaged under process conditions (i.e., serum-free media). Viral DNA extracted from passages 11 and 12 show no evidence of rearrangement.

30



Table 4:  
Amplification Ratios Based on AEX and QPA Analysis of  
Virus Amplification from Passage 3 to Passage 4.

Ad gag construct	Amplification Ratio
MRKAd5gag	470
HCMV-Flgag-bGHpA [E3-]	115
HCMV-Flgag-SPA [E3+]	320
mCMV-FLgag-bGHpA [E3+]	420
Original construct *	40 - 50

\* This estimation is based on the clinical lot growth characteristics at Passage 12.

### EXAMPLE 13

#### Analytical Evaluation of the enhanced Ad5 Constructs

To study the effects of the transgene and the E3 gene on virus amplification, the enhanced adenoviral vector, MRK Ad5 HIV-1 gag, along with its transgene-less version (MRKpAdHVE3) and its E3- version (MRK Ad5 HIV-1 gag E3-), was studied for several passages under serum-free conditions. Table 5A shows the amplification ratios determined for passages P3 to P8 for MRK Ad5 HIV-1 gag. Within a certain MOI range, it has been determined that the virus output is directly proportional to the virus input. Therefore, the greater the number of virus particles per cell at infection, the greater the virus amount produced. Viral amplification ratios, on the other hand, are inversely proportional to the virus input. The lower the virus input, the greater the amplification ratio.

Table 5B shows the amplification rates of the new E3+ vector backbone MRKpAdHVE3. It has a significantly lower rate of amplification compared with the gag transgene containing version. This may be contributed to the larger size MRK Ad5 HIV-1 gag since it contains the transgene. This inclusion of the transgene brings the size of the adenovirus closer to the size of a wild type Ad5 virus. It is well known that adenoviruses amplify best when they are at close to their wild type genomic size.

Wild type Ad5 is 35,935 bp. The MRKpAdHVE3 is 32,905 bp in length. The enhanced adenovector MRK Ad5 HIV-1 gag is 35,453bp (See Figure 14 for vector map; see also Figure 15A-X show the complete pre-adenoviral vector sequence, which includes an additional 2,021 bp of the vector backbone).

- 5           Table 5C shows the amplification rates of the new E3- gag containing virus MRK Ad5 HIV-1 gag E3-. Once again, this virus shows lower growth rate than the enhanced adenoviral vector. This may be attributed to the decreased sized of this virus (due to the E3 gene deletion) compared with wild type Ad5. The MRK Ad5 HIV-1 gag E3- virus is 32,810 bp in length. This can be compared with the wild type
- 10 Ad5 which is 35,935 bp and MRK Ad5 HIV-1 gag which is 35,453 bp in length.

**Table 5A:** Amplification ratios determined by AEX and QPA for MRKAd5gag over several continuous passaging in serum free media. Following P5, two replicate samples were taken (rep-1 and rep-2) and analyzed.

### MRKAd5gag rep1

	Xv (10 <sup>6</sup> cells/ml), Infection	Viability (%), Harvest	Harvest Time h.p.i.	Cell Passage Number	Titer 10 <sup>6</sup> vp/ml culture	Titer 10 <sup>6</sup> vp/cell	OPA 10 <sup>6</sup> TCID <sub>50</sub> /ml	Ratio AEX/QPA	Amplification Ratio	AEX Internal Control
P4	1.49, 81%	0.58, 50%	44	46	6.7	5.9	1.73	50	470 (MOI = 125)	
P5	1.38, 83%	0.68, 47%	48	49	6.7	4.9	1.38	49	170	
P6	1.04, 84%	0.88, 77%	47	48	5.8	5.8	1.42	41	200	
P7	1.50, 84%	0.95, 61%	49.5	50	3.9	1.4	0.97	40	50	
P7	1.09, 87%	0.78, 65%	50	52	5.2	4.7	1.70	81	170	
P8	1.03, 84%	0.88, 84%	47.5	54	9.0	8.7	1.10	82	810	
P9	0.89, 85%	0.89, 73%	47.5	56	4.4	4.9	1.03	43	175	3.12 2.84
P10	1.09, 81%	1.06, 66%	47.5	58	8.0	2.8	1.18	28	100	2.70 2.60
P11	1.18, 88%	0.88, 65%	47	60	3.6	3.0	1.15	31	110	2.70 2.70
P12	0.88, 81%	0.85, 63%	47.5	47	5.4	5.5	1.20	45	200	2.88 2.60
P13	1.00, 88%	0.70, 67%	49	49	5.8	5.8	1.11	52	210	3.18 3.18
P14	1.94, 82%	0.88, 67%	48	53	6.6	4.4			160	3.28 3.27
P15	0.97, 86%	0.64, 66%	47	47	6.9	7.1			250	3.12 2.91

**Table 5B:** Amplification ratios determined by AEX and QPA for MRKHVE3 over several continuous passaging in serum free media. MRKHVE3 is the new vector backbone which does NOT carry a transgene.

### MRKHVE3

	Xv (10 <sup>6</sup> cells/ml), Infection	Viability (%), Harvest	Harvest Time h.p.i.	Cell Passage Number	Titer 10 <sup>6</sup> vp/ml culture	Titer 10 <sup>6</sup> vp/cell	OPA 10 <sup>6</sup> TCID <sub>50</sub> /ml	Ratio AEX/QPA	Amplification Ratio	AEX Internal Control
P4	1.10, 87%	1.28, 78%	49	54	4.1	3.8	1.70	25	300 (MOI = 125)	
P5	0.82, 89%	1.18, 77%	47	48	4.3	4.7	1.24	35	170	
P6	1.55, 88%	1.26, 76%	49.5	50	1.2	0.8	0.58	21	30	
P6	1.09, 87%	1.11, 81%	49	52	4.0	3.6	1.16	84	130	
P7	1.17, 91%	1.22, 91%	47.5	54	3.7	3.2	0.50	74	110	
P8	0.98, 88%	1.41, 83%	48	56	2.1	2.1	0.47	45	75	3.12 2.84
P9	1.20, 89%	1.28, 81%	47.5	58	0.8	0.7	0.28	28	25	2.70 2.60
P10	0.89, 82%	1.63, 86%	47	60	2.3	2.3	0.43	53	50	2.70 2.70
P11	1.07, 86%	1.25, 83%	48	47	2.7	2.5	0.41	66	90	2.88 2.60
P12	0.80, 81%	1.14, 80%	49.5	49	5.9	7.4	0.48	123	250	3.18 3.18
P13	1.86, 85%	1.14, 65%	45.5	53	5.8	3.0			110	3.28 3.27
P14	0.97, 86%	1.03, 98%	46.5	47	9.4	8.7			330	3.12 2.91
P15	0.87, 89%	0.97, 69%	48.5	49	5.3	6.1			218	2.78 2.52

Table 5C. Amplification ratios determined by AEX and QPA for MRKAd5gag(E3-) over several continuous passaging in serum free media. This construct is identical to the MRKAd5gag construct except that this version is DELETED of the E3 gene.

5

### MRKAd5gag(E3-)

	Xv (10 <sup>6</sup> cells/ml), Infection	Viability (%), Harvest	Harvest Time h.p.i.	Cell Passage Number	Titer 10 <sup>10</sup> vp/ml culture	Titer 10 <sup>6</sup> vp/cell	QPA 10 <sup>6</sup> TCID <sub>50</sub> /ml	Ratio AEX:QPA	Amplification Ratio (MOI=125)	AEX Internal Control
P4	1.62, 77%	1.12, 62%	47.5	48	2.0	1.2	0.92	20	100	
P5	1.16, 82%	0.62, 43%	49	49	3.3	2.9	0.99	34	100	
P6	1.71, 86%	0.20, 10%	49	50	4.7	2.7	1.70	28	100	
P6	1.09, 97%	0.63, 54%	49.5	52	5.4	5.0	1.78	31	180	
P7	1.17, 91%	0.98, 72%	47.50	54	7.1	6.1	0.67	106	220	
P8	0.98, 88%	0.77, 48%	48	56	3.1	3.2	0.66	47	115	3.12 2.84
P9	1.20, 89%	1.03, 72%	48	58	1.8	1.5	0.67	32	55	2.70 2.60
P10	0.99, 82%	0.80, 62%	46.5	60	3.2	3.2	0.68	47	115	2.70 2.70
P11	1.07, 96%	0.98, 70%	48.5	47	5.9	5.5	0.68	67	200	2.88 2.60
P12	0.80, 91%	0.67, 59%	50	49	6.1	6.4	0.72	71	230	3.18 3.18
P13	1.96, 95%	0.91, 59%	45.5	53	7.4	3.8			135	3.28 3.27
P14	0.97, 96%	0.81, 74%	48	47	6.8	7.0			250	3.12 2.91
P15	0.87, 89%	0.84, 58%	49	49	4.8	5.5			196	2.78 2.52

### EXAMPLE 14

#### Gag Expression Analysis of the Novel Constructs

- In vitro* gag analysis of the MRK Ad5 HIV-1 gag and the original HIV-gag vectors (research and clinical lot) show comparable gag expression. The clinical lot shows only a slightly reduced gag expression level. The most noticeable difference is with the mCMV vector. This vector shows roughly 3 fold lower expression levels compared with the other vectors tested (which all contain hCMV promoters). The mCMV-FLgag with bGHpA assay was performed three times using different propagation and purification lots and it consistently exhibited weaker gag expression.

### EXAMPLE 15

#### Evaluation of MRK Ad5 HIV-1 gag and Other gag-Containing Adenovectors in Balb/c Mice

- Cohorts of 10 balb/c mice were vaccinated intramuscularly with escalating doses of MRK Ad5 HIV-1 gag, and the research and clinical lots of original Ad5HIV-1gag. Serum samples were collected 3 weeks post dose 1 and analyzed by anti-p24 sandwich ELISA.

Anti-p24 titers in mice that received MRK Ad5 HIV-1 gag (10<sup>7</sup> and 10<sup>9</sup> vp(viral particle) doses) were comparable (Figure 13) to those of the research lot of Ad5HIV-1 gag, for which much of the early rhesus data were generated on. These titers were also comparable when E3 is deleted (MRKAd5hCMVgagbGHpA(E3-)) or SPA is substituted for bGHpA terminator (MRKAd5 hCMV-gag-SPA (E3+)) or murine CMV promoter is used in place of hCMV (MRKAd5 mCMV-gag-bGHpA (E3+)) in the MRKAd5 backbone.

The results shown in Table 7 indicate that the three other vectors ( in addition to the preferred vector, MRK Ad5 HIV-1 gag, are also capable of inducing strong anti-gag antibody responses in mice. Interestingly enough, while the mCMV-FLgag construct containing bGHpA and E3+ in an E1 parallel orientation showed lowest gag expression in the COS cell *in vitro* infection (Table 6) in comparison with the other vectors tested, it generated the greatest anti-gag antibody response this *in vivo* Balb/c study. Table 7 also shows a dose response in anti-gag antibody production in both the research and the clinical lot. As expected, the clinical lot shows reduced anti-gag antibody induction at each dosage level compared to the same dosage used for the research lot.

Table 6: *In vitro* analysis for gag expression in COS cells by Elisa assay.

Viral Vectors <sup>a</sup>	μg gag/4.8x10 <sup>5</sup> COS/10e8 parts/48hr
MRKAd5gag <sup>b</sup>	1.40
Clinical lot Ad5gag <sup>c</sup>	1.28
Research lot Ad5gag <sup>d</sup>	1.32
MCMVFL-gagbGHpA <sup>e</sup>	0.42

<sup>a</sup> A<sub>260nm</sub> absorbance readings taken for viral particle determinations.

<sup>b</sup> MRKAd5gag was produced in serum free conditions and purified at P5.

<sup>c</sup> Clinical lot# Ad5gagFN0001

<sup>d</sup> Research Ad5FLgag lot# 6399

<sup>e</sup> mCMVFL-gagbGHpA was produced in serum free conditions and purified at P5.

**Table 7: mHIV020 Anti-p24 Ab Titers in Balb/c mice (n=10) vaccinated with various Adgag constructs and lots (3 week post dose1).**

Group ID	Vaccine	Dose (vp)	GMT	SE upper	SE lower
1	<sup>a</sup> MRKAd5gag	10 <sup>7</sup>	25600	5877	4780
2	"	10 <sup>9</sup>	409600	94028	76473
3	hCMV FL-gag bGHpA [E3-] →	10 <sup>7</sup>	7352	2077	1620
4	"	10 <sup>9</sup>	235253	59767	47659
5	hCMV FL-gag SPA [E3+] →	10 <sup>7</sup>	12800	9905	236
6	"	10 <sup>9</sup>	310419	99181	75165
7	<sup>b</sup> mCMV FL-gag bGHpA [E3+] →	10 <sup>7</sup>	44572	23504	15389
8	"	10 <sup>9</sup>	941014	239068	190836
9	<sup>c</sup> hCMV FL-gag bGHpA [E3-] ←	10 <sup>7</sup>	3676	934	745
10	"	10 <sup>9</sup>	117627	17491	15227
11	research lot hCMV IntronA FL-gag bGHpA [E3-] <-	10 <sup>6</sup>	528	262	175
12	"	10 <sup>7</sup>	14703	5274	3882
13	"	10 <sup>8</sup>	58813	14942	11915
14	"	10 <sup>9</sup>	204800	53232	42250
15	clinical lot hCMVintronA FL-gag bGHpA [E3-] <-	10 <sup>6</sup>	230	82	61
16	"	10 <sup>7</sup>	4222	3405	1138
17	"	10 <sup>8</sup>	19401	3939	3274
18	"	10 <sup>9</sup>	89144	25187	19639
19	Naïve	none	93	7	6

<sup>a</sup>2x50 µL i.m. (quad) injections/animal

P.I.s: Youil, Chen, Casimiro

Vaccination: T. Toner, Q. Su

Assay: M. Chen

<sup>a</sup>The structure of MRKAd5gag is: hCMVFL-gagbGHpA [E3+] → The same lot of MRKAd5gag used in this rodent study was used in the Rhesus monkey study (Tables 7 and 8).

<sup>b</sup>The same lot of mCMVFL-gagbGHpA[E3+] used in the *in vitro* study (Table 6) was used here.

<sup>c</sup>This construct was designed by Volker Sandig. It contains a shorter version of the hCMV promoter than that used in the MRK constructs. The adenovector backbone is identical to the original backbone used in the original Adgag vector. Expression at 10<sup>6</sup>7 dose from this vector is 7 fold lower than the same dose of the MRKAd5gag and 4 fold lower than the research lot.

## EXAMPLE 16

### Comparison of Humoral and Cellular Responses Towards the Original Ad-gag Construct with the New MRK Ad5 HIV-1 gag in Rhesus Monkeys

- 5 Cohorts of 3 rhesus monkeys were vaccinated intramuscularly with MRK Ad5 HIV-1 gag or the clinical Ad5gag bulk at two doses, 10<sup>11</sup> vp and 10<sup>9</sup> vp. Immunizations were conducted at week 0, 4, and 25. Serum and PBMC samples were collected at selected time points. The serum sample were assayed for anti-p24 Ab titers (using competitive based assay) and the PBMCs for antigen-specific IFN-  
10 gamma secretion following overnight stimulation with gag 20-mer peptide pool (via ELISpot assay).

The results shown in Table 8 indicate comparable responses with respect to the generation of anti-gag antibodies. The frequencies of gag-specific T cells in

- peripheral blood assu summarized in Table 9 demonstrate a strong cellular immune response generated after a single dose with the new construct MRK Ad5 HIV-1 gag. The responses are also boostable with second dose of the same vector. The vector is also able to induce CD8+ T cell responses (as evident by remaining spot counts after
- 5 CD4+ depletion of PBMCs) which are responsible for cytotoxic activity.

Table 8 Anti-p24 antibody titers (in mMU/mL) in rhesus macaques immunized with gag-expressing adenovectors (Protocol HIV203).

Vaccine	Pre	Wk 4	Wk 8	Wk 12	Wk 16	Wk 20	Wk 25	Wk 28
<b>MRKAd5gag<sup>P</sup>, 10<sup>11</sup> vp</b>								
97N010	<10	118	5528	11523	7062	21997	ND	51593
97N116	<10	62	772	1447	1562	2174	ND	20029
98X007	<10	66	3353	6156	6845	3719	ND	24031
<b>MRKAd5gag, 10<sup>9</sup> vp</b>								
97N120	<10	51	204	318	366	482	ND	6550
97N144	<10	18	118	274	706	888	ND	7136
98X008	<10	15	444	386	996	1072	ND	12851
<b>Ad5gag<sup>P</sup>, Clinical Lot, 10<sup>11</sup> vp</b>								
97X001	<10	87	2579	4718	7174	7250	ND	69226
97N146	<10	72	3604	7380	7526	18906	ND	60283
98X009	<10	78	4183	3946	3124	6956	ND	26226
<b>Ad5gag, Clinical Lot, 10<sup>9</sup> vp</b>								
97N020	<10	<10	143	371	390	1821	ND	17177
97X003	<10	<10	39	93	156	596	ND	2053
98X012	<10	81	342	717	956	1558	ND	11861
<sup>P</sup> MRKAd5gag (hCMV, bGHpA, E3+)								
<sup>P</sup> original Ad5gag vector (hCMV/intron A, bGHpA, E3-), lot#FN0001								
ND, not determined								

Table 9. Number of gag-specific T cells per million peripheral blood mononuclear cells (PBMCs) in rhesus monkeys immunized with gag-expressing adenovectors. Also included are those frequencies in PBMCs depleted of CD4<sup>+</sup> T cells.

Grp #	Vaccination T=0,4,25 wks	Monkey ID	T=4 Wk		T=6 Wk		T=11 Wk		T=16 Wk		T=25 Wk		T=28 Wk	
			Media <sup>a</sup>	Gag H <sup>b</sup>	Media	Gag H	Media	Gag H	Media	Gag H	Media	Gag H	Media	Gag H
1	MRKAd5gag 10 <sup>9</sup> 11 vp	97N010	8	89	0	395	0	1058	0	1174	3	775	4	1074
		97N010(CD4-)	4	38			3	993			0	76	0	594
		97N116	1	398	1	609	0	534	4	395	1	261	0	408
		97N116(CD4-)	11	676			0	593			0	184	0	666
		98X007	10	579	0	1304	3	2193	1	2118	3	1588	0	2113
		98X007(CD4-)	20	965			0	2675			0	1656	0	1278
2	MRKAd5gag 10 <sup>9</sup> 9 vp	97N120	5	275	1	249	4	141	4	119	9	206	4	219
		97N120(CD4-)	11	170			0	85			0	75	1	219
		97N144	3	236	6	438	1	318	3	256	1	98	5	373
		97N144(CD4-)	6	148			0	285			ND	ND	0	625
		98X008	4	368	1	1090	3	891	4	673	3	473	5	735
		98X008(CD4-)	14	696			0	1175			0	391	4	848
3	Ad5gag clinical lot 10 <sup>9</sup> 11 vp	97X001	0	281	1	485	0	817	0	1220b	1	894	0	1858
		97X001(CD4-)	10	283			3	996			0	1010	0	1123
		97N146	3	150	1	465	0	339	1	1272	3	1238	3	1785
		97N146(CD4-)	6	133			0	370			0	654	0	971
		98X009	0	93	3	339	3	559	0	896	1	384	0	1748
		98X009(CD4-)	0	73			0	333			0	225	0	644
4	Ad5gag clinical lot 10 <sup>9</sup> 9 vp	97N020	3	30	1	101	0	66	0	36	0	26	0	41
		97N020(CD4-)	10	29			0	15			0	1	0	16
		97X003	4	68	5	184	0	18	1	38	4	38	6	81
		97X003(CD4-)	9	40			0	6			0	4	0	19
		98X012	5	85	3	54	1	34	0	18	0	20	1	121
		98X012(CD4-)	11	70			0	11			0	8	0	41
5	Native	96R041	8	8	1	1	0	0	0	0	0	0	1	0
		053F	14	18	5	18	20	14	19	15	10	15	24	9

Based on either 4x10<sup>6</sup> or 2x10<sup>6</sup> cells per well (depending on spot density)

ND, not determined

<sup>a</sup> mock or no peptide control

<sup>b</sup> Pool of 20-aa peptides overlapping by 10 aa and encompassing the gag sequence

5

The adenovectors described herein and, particularly, MRK Ad5 HIV-1 gag, represent very promising HIV-gag adenovectors with respect to their enhanced growth characteristics in both serum and, more importantly, in serum-free media conditions. In comparison with the current HIV-1 gag adenovector construct, MRK Ad5 HIV-1 gag shows a 5-10 fold increased amplification rate. We have shown that it is genetically stable at passage 21. This construct is able to generate significant cellular immune responses *in vivo* even at a relatively low dose of 10<sup>9</sup> vp. The potency of the MRKAd5gag construct is comparable to, if not better than the original HIV-1gag vector as shown in this rhesus monkey study.

15

#### EXAMPLE 17

#### CODON OPTIMIZED HIV-1 POL AND CODON OPTIMIZED HIV-1 POL MODIFICATIONS

20

The open reading frames for the various synthetic *pol* genes disclosed herein comprise coding sequences for the reverse transcriptase (or RT which consists of a polymerase and RNase H activity) and integrase (IN). The protein sequence is based



on that of Hxb2r, a clonal isolate of IIB; this sequence has been shown to be closest to the consensus clade B sequence with only 16 nonidentical residues out of 848 (Korber, et al., 1998, Human retroviruses and AIDS, Los Alamos National Laboratory, Los Alamos, New Mexico). The skilled artisan will understand after review of this specification that any available HIV-1 or HIV-2 strain provides a potential template for the generation of HIV pol DNA vaccine constructs disclosed herein. It is further noted that the protease gene is excluded from the DNA vaccine constructs of the present invention to insure safety from any residual protease activity in spite of mutational inactivation. The design of the gene sequences for both wild-type (wt-pol) and inactivated pol (IA-pol) incorporates the use of human preferred ("humanized") codons for each amino acid residue in the sequence in order to maximize *in vivo* mammalian expression (Lathe, 1985, *J. Mol. Biol.* 183:1-12). As can be discerned by inspecting the codon usage in SEQ ID NOs: 1, 3, 5 and 7, the following codon usage for mammalian optimization is preferred: Met (ATG), Gly (GGC), Lys (AAG), Trp (TGG), Ser (TCC), Arg (AGG), Val (GTG), Pro (CCC), Thr (ACC), Glu (GAG); Leu (CTG), His (CAC), Ile (ATC), Asn (AAC), Cys (TGC), Ala (GCC), Gln (CAG), Phe (TTC) and Tyr (TAC). For an additional discussion relating to mammalian (human) codon optimization, see WO 97/31115 (PCT/US97/02294), which, as noted elsewhere in this specification, is hereby incorporated by reference. It is intended that the skilled artisan may use alternative versions of codon optimization or may omit this step when generating HIV pol vaccine constructs within the scope of the present invention. Therefore, the present invention also relates to non-codon optimized versions of DNA molecules and associated recombinant adenoviral HIV vaccines which encode the various wild type and modified forms of the HIV Pol protein disclosed herein. However, codon optimization of these constructs is a preferred embodiment of this invention.

A particular embodiment of this portion of the invention comprises codon optimized nucleotide sequences which encode wt-pol DNA constructs (herein, "wt-pol" or "wt-pol (codon optimized)") wherein DNA sequences encoding the protease (PR) activity are deleted, leaving codon optimized "wild type" sequences which encode RT (reverse transcriptase and RNase H activity) and IN integrase activity. A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:1, the open reading frame being contained from an initiating Met residue at nucleotides 10-12 to a termination codon from nucleotides 2560-2562. SEQ ID NO:1 is as follows:

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AGATCTACCA TGGCCCCAT CTCCCCATT GAGACTGTGC CTGTGAAGCT GAAGCCTGGC
ATGGATGGCC CCAAGGTGAA GCAGTGGCCC CTGACTGAGG AGAAGATCAA GGCCCTGGTG

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GAAATCTGCA CTGAGATGGA GAAGGAGGGC AAAATCTCCA AGATTGGCCC CGAGAACCCC  
TACAACACCC CTGTGTTTGC CATCAAGAAG AAGGACTCCA CCAAGTGGAG GAAGCTGGTG  
GACTTCAGGG AGCTGAACAA GAGGACCCAG GACTTCTGGG AGGTGCAGCT GGGCATCCCC  
CACCCCGCTG GCCTGAAGAA GAAGAAGTCT GTGACTGTGC TGGATGTGGG GGATGCCTAC  
5 TTCTCTGTGC CCCTGGATGA GGACTTCAGG AAGTACACTG CCTTCACCAT CCCCTCCATC  
AACAATGAGA CCCCTGGCAT CAGGTACCAG TACAATGTGC TGCCCCAGGG CTGGAAGGGC  
TCCCCTGCCA TCTTCCAGTC CTCCATGACC AAGATCCTGG AGCCCTTCAG GAAGCAGAAC  
CCTGACATTG TGATCTACCA GTACATGGAT GACCTGTATG TGGGCTCTGA CCTGGAGATT  
GGGCAGCACA GGACCAAGAT TGAGGAGCTG AGGCAGCACC TGCTGAGGTG GGGCCTGACC  
10 ACCCCTGACA AGAAGCACCA GAAGGAGCCC CCCTTCCTGT GGATGGGCTA TGAGCTGCAC  
CCCGACAAGT GGACTGTGCA GCCCATTGTG CTGCCCTGAGA AGGACTCCTG GACTGTGAAT  
GACATCCAGA AGCTGGTGGG CAAGCTGAAC TGGGCCCTCC AAATCTACCC TGGCATCAAG  
GTGAGGCAGC TGTGCAAGCT GCTGAGGGGC ACCAAGGCCC TGACTGAGGT GATCCCCCTG  
ACTGAGGAGG CTGAGCTGGA GCTGGCTGAG AACAGGGAGA TCCTGAAGGA GCCTGTGCAT  
15 GGGGTGTACT ATGACCCCTC CAAGGACCTG ATTGCTGAGA TCCAGAAGCA GGGCCAGGGC  
CAGTGGACCT ACCAAATCTA CCAGGAGCCC TTCAAGAACC TGAAGACTGG CAAGTATGCC  
AGGATGAGGG GGGCCACAC CAATGATGTG AAGCAGCTGA CTGAGGCTGT GCAGAAGATC  
ACCACTGAGT CCATTGTGAT CTGGGGCAAG ACCCCCAAGT TCAAGCTGCC CATCCAGAAG  
GAGACCTGGG AGACCTGGTG GACTGAGTAC TGGCAGGCCA CCTGGATCCC TGAGTGGGAG  
20 TTTGTGAACA CCCCCCCCCT GGTGAAGCTG TGGTACCAGC TGGAGAAGGA GCCCATTTGTG  
GGGGCTGAGA CTTCTATGT GGATGGGGCT GCCAACAGGG AGACCAAGCT GGGCAAGGCT  
GGCTATGTGA CCAACAGGGG CAGGCAGAAG GTGGTGACCC TGACTGACAC CACCAACCAG  
AAGACTGAGC TCCAGGCCAT CTACCTGGCC CTCCAGGACT CTGGCCTGGA GGTGAACATT  
GTGACTGACT CCCAGTATGC CCTGGGCATC ATCCAGGCCC AGCCTGATCA GTCTGAGTCT  
25 GAGCTGGTGA ACCAGATCAT TGAGCAGCTG ATCAAGAAGG AGAAGGTGTA CCTGGCCTGG  
GTGCCCTGCCC ACAAGGGCAT TGGGGGCAAT GAGCAGGTGG ACAAGCTGGT GTCTGTCTGGC  
ATCAGGAAGG TGCTGTTTCT GGATGGCATT GACAAGGCCC AGGATGAGCA TGAGAAGTAC  
CACTCCAACCT GGAGGGCTAT GGCCTCTGAC TTCAACCTGC CCCCTGTGGT GGCTAAGGAG  
ATTGTGGCCT CCTGTGACAA GTGCCAGCTG AAGGGGGAGG CCATGCATGG GCAGGTGGAC  
30 TGCTCCCCCTG GCATCTGGCA GCTGGACTGC ACCCACCTGG AGGGCAAGGT GATCCTGGTG  
GCTGTGCATG TGGCCTCCGG CTACATTGAG GCTGAGGTGA TCCCTGCTGA GACAGGCCAG  
GAGACTGCCT ACTTCCTGCT GAAGCTGGCT GGCAGGTGGC CTGTGAAGAC CATCCACACT  
GACAATGGCT CCAACTTCAC TGGGGCCACA GTGAGGGCTG CCTGCTGGTG GGCTGGCATC  
AAGCAGGAGT TTGGCATCCC CTACAACCCC CAGTCCCAGG GGGTGGTGGA GTCCATGAAC  
35 AAGGAGCTGA AGAAGATCAT TGGGCAGGTG AGGGACCAGG CTGAGCACCT GAAGACAGCT  
GTGCAGATGG CTGTGTTTCT CCACAACCTC AAGAGGAAGG GGGGCATCGG GGGCTACTCC

GCTGGGGAGA GGATTGTGGA CATCATTGCC ACAGACATCC AGACCAAGGA GCTCCAGAAG  
 CAGATCACCA AGATCCAGAA CTTCAGGGTG TACTACAGGG ACTCCAGGAA CCCCCTGTGG  
 AAGGGCCCTG CCAAGCTGCT GTGGAAGGGG GAGGGGGCTG TGGTGATCCA GGACAACTCT  
 GACATCAAGG TGGTGCCAG GAGGAAGGCC AAGATCATCA GGGACTATGG CAAGCAGATG  
 5 GCTGGGGATG ACTGTGTGGC CTCCAGGCAG GATGAGGACT AAAGCCCGGG CAGATCT (SEQ  
 ID NO:1).

The open reading frame of the wild type pol construct disclosed as SEQ ID  
 NO:1 contains 850 amino acids, disclosed herein as SEQ ID NO:2, as follows:

Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro  
 10 Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys  
 Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys  
 Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala  
 Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg  
 Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile  
 15 Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Asp  
 Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys  
 Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile  
 Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala  
 Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln  
 20 Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Asp Asp Leu Tyr Val Gly  
 Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg  
 Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln  
 Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys  
 Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val  
 25 Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile  
 Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr  
 Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu  
 Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr  
 Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln  
 30 Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys  
 Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys  
 Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile  
 Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp  
 Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp  
 35 Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu  
 Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Asp Gly Ala Ala

Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly  
 Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Glu  
 Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn  
 Ile Val Thr Asp Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro  
 5 Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile  
 Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile  
 Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys  
 Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys  
 Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro  
 10 Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys  
 Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln  
 Leu Asp Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His  
 Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly  
 Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val  
 15 Lys Thr Ile His Thr Asp Asn Gly Ser Asn Phe Thr Gly Ala Thr Val  
 Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro  
 Tyr Asn Pro Gln Ser Gln Gly Val Val Glu Ser Met Asn Lys Glu Leu  
 Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr  
 Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly  
 20 Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr  
 Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn  
 Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro  
 Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn  
 Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp  
 25 Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp  
 Glu Asp (SEQ ID NO:2).

The present invention especially relates to an adenoviral vector vaccine which  
 comprises a codon optimized HIV-1 DNA pol construct wherein, in addition to  
 deletion of the portion of the wild type sequence encoding the protease activity, a  
 30 combination of active site residue mutations are introduced which are deleterious to  
 HIV-1 pol (RT-RH-IN) activity of the expressed protein. Therefore, the present  
 invention preferably relates to an adenoviral HIV-1 DNA pol-based vaccine wherein  
 the construct is devoid of DNA sequences encoding any PR activity, as well as  
 containing a mutation(s) which at least partially, and preferably substantially,  
 35 abolishes RT, RNase and/or IN activity. One type of HIV-1 pol mutant which is part  
 and parcel of an adenoviral vector vaccine may include but is not limited to a mutated

DNA molecule comprising at least one nucleotide substitution which results in a point mutation which effectively alters an active site within the RT, RNase and/or IN regions of the expressed protein, resulting in at least substantially decreased enzymatic activity for the RT, RNase H and/or IN functions of HIV-1 Pol. In a preferred embodiment of this portion of the invention, a HIV-1 DNA pol construct contains a mutation or mutations within the Pol coding region which effectively abolishes RT, RNase H and IN activity. An especially preferable HIV-1 DNA pol construct in a DNA molecule which contains at least one point mutation which alters the active site of the RT, RNase H and IN domains of Pol, such that each activity is at least substantially abolished. Such a HIV-1 Pol mutant will most likely comprise at least one point mutation in or around each catalytic domain responsible for RT, RNase H and IN activity, respectfully. To this end, an especially preferred HIV-1 DNA pol construct is exemplified herein and contains nine codon substitution mutations which results in an inactivated Pol protein (IA Pol: SEQ ID NO:4, Figure 17A-C) which has no PR, RT, RNase or IN activity, wherein three such point mutations reside within each of the RT, RNase and IN catalytic domains. Therefore, an especially preferred exemplification is an adenoviral vaccine which comprises, in an appropriate fashion, a DNA molecule which encodes IA-pol, which contains all nine mutations as shown below in Table 1. An additional preferred amino acid residue for substitution is Asp551, localized within the RNase domain of Pol. Any combination of the mutations disclosed herein may suitable and therefore may be utilized as an IA-Pol-based vaccine of the present invention. While addition and deletion mutations are contemplated and within the scope of the invention, the preferred mutation is a point mutation resulting in a substitution of the wild type amino acid with an alternative amino acid residue.

Table 1

	<u>wt aa</u>	<u>aa residue</u>	<u>mutant aa</u>	<u>enzyme function</u>
	Asp	112	Ala	RT
	Asp	187	Ala	RT
30	Asp	188	Ala	RT
	Asp	445	Ala	RNase H
	Glu	480	Ala	RNase H
	Asp	500	Ala	RNase H
	Asp	626	Ala	IN
35	Asp	678	Ala	IN
	Glu	714	Ala	IN

It is preferred that point mutations be incorporated into the IApol mutant adenoviral vaccines of the present invention so as to lessen the possibility of altering epitopes in and around the active site(s) of HIV-1 Pol.

To this end, SEQ ID NO:3 discloses the nucleotide sequence which codes for a codon optimized pol in addition to the nine mutations shown in Table 1, disclosed as follows, and referred to herein as "IApol":

```

AGATCTACCA TGGCCCCCAT CTCCCCATT GAGACTGTGC CTGTGAAGCT GAAGCCTGGC
ATGGATGGCC CCAAGGTGAA GCAGTGGCCC CTGACTGAGG AGAAGATCAA GGCCCTGGTG
GAAATCTGCA CTGAGATGGA GAAGGAGGGC AAAATCTCCA AGATTGGCCC CGAGAACCCC
10 TACAACACCC CTGTGTTTGC CATCAAGAAG AAGGACTCCA CCAAGTGGAG GAAGCTGGTG
GACTTCAGGG AGCTGAACAA GAGGACCCAG GACTTCTGGG AGGTGCAGCT GGGCATCCCC
CACCCCGCTG GCCTGAAGAA GAAGAAGTCT GTGACTGTGC TGGCTGTGGG GGATGCCTAC
TTCTCTGTGC CCCTGGATGA GGACTTCAGG AAGTACACTG CCTTCACCAT CCCCTCCATC
AACAATGAGA CCCCTGGCAT CAGGTACCAG TACAATGTGC TGCCCCAGGG CTGGAAGGGC
15 TCCCCTGCCA TCTTCCAGTC CTCCATGACC AAGATCCTGG AGCCCTTCAG GAAGCAGAAC
CCTGACATTG TGATCTACCA GTACATGGCT GCCCTGTATG TGGGCTCTGA CCTGGAGATT
GGGCAGCACA GGACCAAGAT TGAGGAGCTG AGGCAGCACC TGCTGAGGTG GGGCCTGACC
ACCCCTGACA AGAAGCACCA GAAGGAGCCC CCCTTCCTGT GGATGGGCTA TGAGCTGCAC
CCCGACAAGT GGACTGTGCA GCCCATTTGT CTGCCTGAGA AGGACTCCTG GACTGTGAAT
20 GACATCCAGA AGCTGGTGGG CAAGCTGAAC TGGGCCCTCC AAATCTACCC TGGCATCAAG
GTGAGGCAGC TGTGCAAGCT GCTGAGGGGC ACCAAGGCCC TGACTGAGGT GATCCCCCTG
ACTGAGGAGG CTGAGCTGGA GCTGGCTGAG AACAGGGAGA TCCTGAAGGA GCCTGTGCAT
GGGGTGTACT ATGACCCCTC CAAGGACCTG ATTGCTGAGA TCCAGAAGCA GGGCCAGGGC
CAGTGGACCT ACCAAATCTA CCAGGAGCCC TTCAAGAACC TGAAGACTGG CAAGTATGCC
25 AGGATGAGGG GGGCCACAC CAATGATGTG AAGCAGCTGA CTGAGGCTGT GCAGAAGATC
ACCACTGAGT CCATTGTGAT CTGGGGCAAG ACCCCCAAGT TCAAGCTGCC CATCCAGAAG
GAGACCTGGG AGACCTGGTG GACTGAGTAC TGGCAGGCCA CCTGGATCCC TGAGTGGGAG
TTTGTGAACA CCCCCCCCCT GGTGAAGCTG TGGTACCAGC TGGAGAAGGA GCCCATTTGTG
GGGGCTGAGA CCTTCTATGT GGCTGGGGCT GCCAACAGGG AGACCAAGCT GGGCAAGGCT
30 GGCTATGTGA CCAACAGGGG CAGGCAGAAG GTGGTGACCC TGACTGACAC CACCAACCAG
AAGACTGCCC TCCAGGCCAT CTACCTGGCC CTCCAGGACT CTGGCCTGGA GGTGAACATT
GTGACTGCCT CCCAGTATGC CCTGGGCATC ATCCAGGCCC AGCCTGATCA GTCTGAGTCT
GAGCTGGTGA ACCAGATCAT TGAGCAGCTG ATCAAGAAGG AGAAGGTGTA CCTGGCCTGG
GTGCCTGCCC ACAAGGGCAT TGGGGGCAAT GAGCAGGTGG ACAAGCTGGT GTCTGCTGGC
35 ATCAGGAAGG TGCTGTTCCCT GGATGGCATT GACAAGGCCC AGGATGAGCA TGAGAAGTAC
CACTCCAACCT GGAGGGCTAT GGCCTCTGAC TTCAACCTGC CCCCTGTGGT GGCTAAGGAG

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ATTGTGGCCT CCTGTGACAA GTGCCAGCTG AAGGGGGAGG CCATGCATGG GCAGGTGGAC  
 TGCTCCCCCTG GCATCTGGCA GCTGGCCTGC ACCCACCTGG AGGGCAAGGT GATCCTGGTG  
 GCTGTGCATG TGGCCTCCGG CTACATTGAG GCTGAGGTGA TCCCTGCTGA GACAGGCCAG  
 GAGACTGCCT ACTTCCTGCT GAAGCTGGCT GGCAGGTGGC CTGTGAAGAC CATCCACACT  
 5 GCCAATGGCT CCAACTTCAC TGGGGCCACA GTGAGGGCTG CCTGCTGGTG GGCTGGCATC  
 AAGCAGGAGT TTGGCATCCC CTACAACCCC CAGTCCCAGG GGGTGGTGGC CTCCATGAAC  
 AAGGAGCTGA AGAAGATCAT TGGGCAGGTG AGGGACCAGG CTGAGCACCT GAAGACAGCT  
 GTGCAGATGG CTGTGTTTAT CCACAACCTT AAGAGGAAGG GGGGCATCGG GGGCTACTCC  
 GCTGGGGAGA GGATTGTGGA CATCATTGCC ACAGACATCC AGACCAAGGA GCTCCAGAAG  
 10 CAGATCACCA AGATCCAGAA CTTCAGGGTG TACTACAGG ACTCCAGGAA CCCCCTGTGG  
 AAGGGCCCTG CCAAGCTGCT GTGGAAGGGG GAGGGGGCTG TGGTGATCCA GGACAACCTCT  
 GACATCAAGG TGGTGGCCAG GAGGAAGGCC AAGATCATCA GGGACTATGG CAAGCAGATG  
 GCTGGGGATG ACTGTGTGGC CTCCAGGCAG GATGAGGACT AAAGCCCGGG CAGATCT (SEQ ID  
 NO:3).

15 In order to produce the IA-pol-based adenoviral vaccines of the present  
 invention, inactivation of the enzymatic functions was achieved by replacing a total of  
 nine active site residues from the enzyme subunits with alanine side-chains. As  
 shown in Table 1, all residues that comprise the catalytic triad of the polymerase,  
 namely Asp112, Asp187, and Asp188, were substituted with alanine (Ala) residues  
 20 (Larder, et al., *Nature* 1987, 327: 716-717; Larder, et al., 1989, *Proc. Natl. Acad. Sci.*  
 1989, 86: 4803-4807). Three additional mutations were introduced at Asp445,  
 Glu480 and Asp500 to abolish RNase H activity (Asp551 was left unchanged in this  
 IA Pol construct), with each residue being substituted for an Ala residue, respectively  
 (Davies, et al., 1991, *Science* 252:, 88-95; Schatz, et al., 1989, *FEBS Lett.* 257: 311-  
 25 314; Mizrahi, et al., 1990, *Nucl. Acids. Res.* 18: pp. 5359-5353). HIV pol integrase  
 function was abolished through three mutations at Asp626, Asp678 and Glu714.  
 Again, each of these residues has been substituted with an Ala residue (Wiskerchen,  
 et al., 1995, *J. Virol.* 69: 376-386; Leavitt, et al., 1993, *J. Biol. Chem.* 268: 2113-  
 2119). Amino acid residue Pro3 of SEQ ID NO:4 marks the start of the RT gene.  
 30 The complete amino acid sequence of IA-Pol is disclosed herein as SEQ ID NO:4 and  
 Figure 17A-C, as follows:

Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro  
 Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys  
 Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys  
 35 Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala  
 Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg

Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile  
 Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Ala  
 Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys  
 Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile  
 5 Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala  
 Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln  
 Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Ala Ala Leu Tyr Val Gly  
 Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg  
 Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln  
 10 Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys  
 Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val  
 Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile  
 Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr  
 Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu  
 15 Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr  
 Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln  
 Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys  
 Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys  
 Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile  
 20 Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp  
 Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp  
 Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu  
 Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala  
 Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly  
 25 Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Ala  
 Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn  
 Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro  
 Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile  
 Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile  
 30 Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys  
 Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys  
 Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro  
 Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys  
 Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln  
 35 Leu Ala Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His  
 Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly



Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val  
 Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val  
 Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro  
 Tyr Asn Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu  
 5 Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr  
 Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly  
 Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr  
 Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn  
 Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro  
 10 Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn  
 Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp  
 Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp  
 Glu Asp (SEQ ID NO:4).

As noted above, it will be understood that any combination of the mutations  
 15 disclosed above may be suitable and therefore be utilized as an IA-pol-based  
 adenoviral HIV vaccine of the present invention, either when administered alone or in  
 a combined modality regime and/or a prime-boost regimen. For example, it may be  
 possible to mutate only 2 of the 3 residues within the respective reverse transcriptase,  
 RNase-H, and integrase coding regions while still abolishing these enzymatic  
 20 activities. However, the IA-pol construct described above and disclosed as SEQ ID  
 NO:3, as well as the expressed protein (SEQ ID NO:4;) is preferred. It is also  
 preferred that at least one mutation be present in each of the three catalytic domains.

Another aspect of this portion of the invention are codon optimized HIV-1  
 Pol-based vaccine constructions which comprise a eukaryotic trafficking signal  
 25 peptide such as from tPA (tissue-type plasminogen activator) or by a leader peptide  
 such as is found in highly expressed mammalian proteins such as immunoglobulin  
 leader peptides. Any functional leader peptide may be tested for efficacy. However,  
 a preferred embodiment of the present invention, as with HIV-1 Nef constructs shown  
 herein, is to provide for a HIV-1 Pol mutant adenoviral vaccine construction wherein  
 30 the pol coding region or a portion thereof is operatively linked to a leader peptide,  
 preferably a leader peptide from human tPA. In other words, a codon optimized  
 HIV-1 Pol mutant such as IA-Pol (SEQ ID NO:4) may also comprise a leader peptide  
 at the amino terminal portion of the protein, which may effect cellular trafficking and  
 hence, immunogenicity of the expressed protein within the host cell. As noted in  
 35 Figure 16A-B, a DNA vector which may be utilized to practice the present invention  
 may be modified by known recombinant DNA methodology to contain a leader signal

peptide of interest, such that downstream cloning of the modified HIV-1 protein of interest results in a nucleotide sequence which encodes a modified HIV-1 tPA/Pol protein. In the alternative, as noted above, insertion of a nucleotide sequence which encodes a leader peptide may be inserted into a DNA vector housing the open reading frame for the Pol protein of interest. Regardless of the cloning strategy, the end result is a polynucleotide vaccine which comprises vector components for effective gene expression in conjunction with nucleotide sequences which encode a modified HIV-1 Pol protein of interest, including but not limited to a HIV-1 Pol protein which contains a leader peptide. The amino acid sequence of the human tPA leader utilized herein is as follows: MDAMKRGGLCCVLLLCGAVFVSPSEISS (SEQ ID NO:17). Therefore, another aspect of the present invention is to generate HIV-1 Pol-based vaccine constructions which comprise a eukaryotic trafficking signal peptide such as from tPA. To this end, the present invention relates to a DNA molecule which encodes a codon optimized wt-pol DNA construct wherein the protease (PR) activity is deleted and a human tPA leader sequence is fused to the 5' end of the coding region. A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:5, the open reading frame disclosed herein as SEQ ID NO:6.

To this end, the present invention relates to a DNA molecule which encodes a codon optimized wt-pol DNA construct wherein the protease (PR) activity is deleted and a human tPA leader sequence is fused to the 5' end of the coding region ( herein, "tPA-wt-pol"). A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:5, the open reading frame being contained from an initiating Met residue at nucleotides 8-10 to a termination codon from nucleotides 2633-2635. SEQ ID NO:5 is as follows:

25 GATCACCATG GATGCAATGA AGAGAGGGCT CTGCTGTGTG CTGCTGCTGT GTGGAGCAGT  
CTTCGTTTCG CCCAGCGAGA TCTCCGCCCC CATCTCCCCC ATTGAGACTG TGCCTGTGAA  
GCTGAAGCCT GGCATGGATG GCCCAAGGT GAAGCAGTGG CCCCTGACTG AGGAGAAGAT  
CAAGGCCCTG GTGGAAATCT GCACTGAGAT GGAGAAGGAG GGCAAAATCT CCAAGATTGG  
CCCCGAGAAC CCCTACAACA CCCCTGTGTT TGCCATCAAG AAGAAGGACT CCACCAAGTG  
30 GAGGAAGCTG GTGGACTTCA GGGAGCTGAA CAAGAGGACC CAGGACTTCT GGGAGGTGCA  
GCTGGGCATC CCCACCCCG CTGGCCTGAA GAAGAAGAAG TCTGTGACTG TGCTGGATGT  
GGGGGATGCC TACTTCTCTG TGCCCTTGGG TGAGGACTTC AGGAAGTACA CTGCCCTTAC  
CATCCCCTCC ATCAACAATG AGACCCCTGG CATCAGGTAC CAGTACAATG TGCTGCCCCA  
GGGCTGGAAG GGCTCCCTCG CCATCTTCCA GTCCTCCATG ACCAAGATCC TGGAGCCCTT  
35 CAGGAAGCAG AACCCTGACA TTGTGATCTA CCAGTACATG GATGACCTGT ATGTGGGCTC  
TGACCTGGAG ATTGGGCAGC ACAGGACCAA GATTGAGGAG CTGAGGCAGC ACCTGCTGAG

GTGGGGCCTG ACCACCCCTG ACAAGAAGCA CCAGAAGGAG CCCCCCTTCC TGTGGATGGG  
 CTATGAGCTG CACCCCGACA AGTGGACTGT GCAGCCCAT TGTGCTGCCTG AGAAGGACTC  
 CTGGACTGTG AATGACATCC AGAAGCTGGT GGGCAAGCTG AACTGGGCCT CCCAAATCTA  
 CCCTGGCATC AAGGTGAGGC AGCTGTGCAA GCTGCTGAGG GGCACCAAGG CCCTGACTGA  
 5 GGTGATCCCC CTGACTGAGG AGGCTGAGCT GGAGCTGGCT GAGAACAGGG AGATCCTGAA  
 GGAGCCTGTG CATGGGGTGT ACTATGACCC CTCCAAGGAC CTGATTGCTG AGATCCAGAA  
 GCAGGGCCAG GGCCAGTGGA CCTACCAAAT CTACCAGGAG CCCTTCAAGA ACCTGAAGAC  
 TGGCAAGTAT GCCAGGATGA GGGGGGCCCA CACCAATGAT GTGAAGCAGC TGACTGAGGC  
 TGTGCAGAAG ATCACCCTG AGTCCATTGT GATCTGGGGC AAGACCCCA AGTTCAAGCT  
 10 GCCCATCCAG AAGGAGACCT GGGAGACCTG GTGGACTGAG TACTGGCAGG CCACCTGGAT  
 CCCTGAGTGG GAGTTTGTGA ACACCCCCC CCTGGTGAAG CTGTGGTACC AGCTGGAGAA  
 GGAGCCCATT GTGGGGGCTG AGACCTTCTA TGTGGATGGG GCTGCCAACA GGGAGACCAA  
 GCTGGGCAAG GCTGGCTATG TGACCAACAG GGGCAGGCAG AAGGTGGTGA CCCTGACTGA  
 CACCACCAAC CAGAAGACTG AGCTCCAGGC CATCTACCTG GCCCTCCAGG ACTCTGGCCT  
 15 GGAGGTGAAC ATTGTGACTG ACTCCCAGTA TGCCCTGGGC ATCATCCAGG CCCAGCCTGA  
 TCAGTCTGAG TCTGAGCTGG TGAACCAGAT CATTGAGCAG CTGATCAAGA AGGAGAAGGT  
 GTACCTGGCC TGGGTGCCTG CCCACAAGGG CATTGGGGGC AATGAGCAGG TGGACAAGCT  
 GGTGTCTGCT GGCATCAGGA AGGTGCTGTT CCTGGATGGC ATTGACAAGG CCCAGGATGA  
 GCATGAGAAG TACCACTCCA ACTGGAGGGC TATGGCCTCT GACTTCAACC TGCCCCCTGT  
 20 GGTGGCTAAG GAGATTGTGG CCTCCTGTGA CAAGTGCCAG CTGAAGGGGG AGGCCATGCA  
 TGGGCAGGTG GACTGCTCCC CTGGCATCTG GCAGCTGGAC TGCACCCACC TGGAGGGCAA  
 GGTGATCCTG GTGGCTGTGC ATGTGGCCTC CGGCTACATT GAGGCTGAGG TGATCCCTGC  
 TGAGACAGGC CAGGAGACTG CCTACTTCCT GCTGAAGCTG GCTGGCAGGT GGCCTGTGAA  
 GACCATCCAC ACTGACAATG GCTCCAATT CACTGGGGCC ACAGTGAGGG CTGCCTGCTG  
 25 GTGGGCTGGC ATCAAGCAGG AGTTTGGCAT CCCCTACAAC CCCCAGTCCC AGGGGGTGGT  
 GGAGTCCATG AACAAGGAGC TGAAGAAGAT CATTGGGCAG GTGAGGGACC AGGCTGAGCA  
 CCTGAAGACA GCTGTGCAGA TGGCTGTGTT CATCCACAAC TTCAAGAGGA AGGGGGGCAT  
 CGGGGGCTAC TCCGCTGGGG AGAGGATTGT GGACATCATT GCCACAGACA TCCAGACCAA  
 GGAGCTCCAG AAGCAGATCA CCAAGATCCA GAACTTCAGG GTGTACTACA GGGACTCCAG  
 30 GAACCCCTG TGGGAAGGGC CTGCCAAGCT GCTGTGGAAG GGGGAGGGGG CTGTGGTGAT  
 CCAGGACAAC TCTGACATCA AGGTGGTGCC CAGGAGGAAG GCCAAGATCA TCAGGGACTA  
 TGGCAAGCAG ATGGCTGGGG ATGACTGTGT GGCCTCCAGG CAGGATGAGG ACTAAAGCCC  
 GGGCAGATCT (SEQ ID NO:5).

The open reading frame of the wild type tPA-pol construct disclosed as SEQ  
 35 ID NO:5 contains 875 amino acids, disclosed herein as SEQ ID NO:6, as follows:  
 Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly

Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ala Pro Ile Ser Pro Ile  
 Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val  
 Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile  
 Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu  
 5 Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr  
 Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln  
 Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys  
 Lys Lys Lys Ser Val Thr Val Leu Asp Val Gly Asp Ala Tyr Phe Ser  
 Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro  
 10 Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu  
 Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr  
 Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr  
 Gln Tyr Met Asp Asp Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln  
 His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly  
 15 Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp  
 Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val  
 Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val  
 Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg  
 Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile  
 20 Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile  
 Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu  
 Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile  
 Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met  
 Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln  
 25 Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe  
 Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr  
 Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro  
 Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala  
 Glu Thr Phe Tyr Val Asp Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly  
 30 Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu  
 Thr Asp Thr Thr Asn Gln Lys Thr Glu Leu Gln Ala Ile Tyr Leu Ala  
 Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Asp Ser Gln Tyr  
 Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu  
 Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu  
 35 Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp  
 Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile

Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala  
 Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val  
 Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln  
 Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Asp Cys Thr His Leu Glu  
 5 Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu  
 Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu  
 Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Asp Asn  
 Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala  
 Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly  
 10 Val Val Glu Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val  
 Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe  
 Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly  
 Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu  
 Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp  
 15 Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly  
 Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro  
 Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly  
 Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:6) .

The present invention also relates to a codon optimized HIV-1 Pol mutant  
 20 contained within a recombinant adenoviral vector such as IA-Pol (SEQ ID NO:4)  
 which comprises a leader peptide at the amino terminal portion of the protein, which  
 may effect cellular trafficking and hence, immunogenicity of the expressed protein  
 within the host cell. Any such adenoviral-based HIV-1 DNA pol mutant disclosed in  
 the above paragraphs is suitable for fusion downstream of a leader peptide, such as a  
 25 leader peptide including but not limited to the human tPA leader sequence. Therefore,  
 any such leader peptide-based HIV-1 pol mutant construct may include but is not  
 limited to a mutated DNA molecule which effectively alters the catalytic activity of  
 the RT, RNase and/or IN region of the expressed protein, resulting in at least  
 substantially decreased enzymatic activity one or more of the RT, RNase H and/or IN  
 30 functions of HIV-1 Pol. In a preferred embodiment of this portion of the invention, a  
 leader peptide/HIV-1 DNA pol construct contains a mutation or mutations within the  
 Pol coding region which effectively abolishes RT, RNase H and IN activity. An  
 especially preferable HIV-1 DNA pol construct is a DNA molecule which contains at  
 least one point mutation which alters the active site and catalytic activity within the  
 35 RT, RNase H and IN domains of Pol, such that each activity is at least substantially  
 abolished, and preferably totally abolished. Such a HIV-1 Pol mutant will most likely

comprise at least one point mutation in or around each catalytic domain responsible for RT, RNase H and IN activity, respectfully. An especially preferred embodiment of this portion of the invention relates to a human tPA leader fused to the IA-Pol protein comprising the nine mutations shown in Table 1. The DNA molecule is disclosed  
 5 herein as SEQ ID NO:7 and the expressed tPA-IA Pol protein comprises a fusion junction as shown in Figure 18. The complete amino acid sequence of the expressed protein is set forth in SEQ ID NO:8. To this end, SEQ ID NO:7 discloses the nucleotide sequence which codes for a human tPA leader fused to the IA Pol protein comprising the nine mutations shown in Table 1 (herein, "tPA-opt-IApol"). The open  
 10 reading frame begins with the initiating Met (nucleotides 8-10) and terminates with a "TAA" codon at nucleotides 2633-2635. The nucleotide sequence encoding tPA-IAPol is also disclosed as follows:

GATCACCATG GATGCAATGA AGAGAGGGCT CTGCTGTGTG CTGCTGCTGT GTGGAGCAGT  
 CTTTCGTTTCG CCCAGCGAGA TCTCCGCCCC CATCTCCCCC ATTGAGACTG TGCCTGTGAA  
 15 GCTGAAGCCT GGCATGGATG GCCCCAAGGT GAAGCAGTGG CCCCTGACTG AGGAGAAGAT  
 CAAGGCCCTG GTGGAAATCT GCACTGAGAT GGAGAAGGAG GGCAAAATCT CCAAGATTGG  
 CCCCAGAGAAC CCCTACAACA CCCCTGTGTT TGCCATCAAG AAGAAGGACT CCACCAAGTG  
 GAGGAAGCTG GTGGACTTCA GGGAGCTGAA CAAGAGGACC CAGGACTTCT GGGAGGTGCA  
 GCTGGGCATC CCCCACCCCG CTGGCCTGAA GAAGAAGAAG TCTGTGACTG TGCTGGCTGT  
 20 GGGGGATGCC TACTTCTCTG TGCCCCCTGGA TGAGGACTTC AGGAAGTACA CTGCCTTCAC  
 CATCCCCCTCC ATCAACAATG AGACCCCTGG CATCAGGTAC CAGTACAATG TGCTGCCCCA  
 GGGCTGGAAG GGCTCCCTG CCATCTTCCA GTCCCTCCATG ACCAAGATCC TGGAGCCCTT  
 CAGGAAGCAG AACCCTGACA TTGTGATCTA CCAGTACATG GCTGCCCTGT ATGTGGGCTC  
 TGACCTGGAG ATTGGGCAGC ACAGGACCAA GATTGAGGAG CTGAGGCAGC ACCTGCTGAG  
 25 GTGGGGCCTG ACCACCCCTG ACAAGAAGCA CCAGAAGGAG CCCCCCTTCC TGTGGATGGG  
 CTATGAGCTG CACCCCGACA AGTGGACTGT GCAGCCCATT GTGCTGCCTG AGAAGGACTC  
 CTGGACTGTG AATGACATCC AGAAGCTGGT GGGCAAGCTG AACTGGGCCT CCCAAATCTA  
 CCCTGGCATC AAGGTGAGGC AGCTGTGCAA GCTGCTGAGG GGCACCAAGG CCCTGACTGA  
 GGTGATCCCC CTGACTGAGG AGGCTGAGCT GGAGCTGGCT GAGAACAGGG AGATCCTGAA  
 30 GGAGCCTGTG CATGGGGTGT ACTATGACCC CTCCAAGGAC CTGATTGCTG AGATCCAGAA  
 GCAGGGCCAG GGCCAGTGGA CCTACCAAT CTACCAGGAG CCCTTCAAGA ACCTGAAGAC  
 TGGCAAGTAT GCCAGGATGA GGGGGGCCCA CACCAATGAT GTGAAGCAGC TGAAGGAGC  
 TGTGCAGAAG ATCACCCTG AGTCCATTGT GATCTGGGGC AAGACCCCA AGTTCAAGCT  
 GCCCATCCAG AAGGAGACCT GGGAGACCTG GTGGACTGAG TACTGGCAGG CCACCTGGAT  
 35 CCCTGAGTGG GAGTTTGTGA ACACCCCCC CCTGGTGAAG CTGTGGTACC AGCTGGAGAA  
 GGAGCCCATT GTGGGGGCTG AGACCTTCTA TGTGGCTGGG GCTGCCAACA GGGAGACCAA

GCTGGGCAAG GCTGGCTATG TGACCAACAG GGGCAGGCAG AAGGTGGTGA CCCTGACTGA  
 CACCACCAAC CAGAAGACTG CCCTCCAGGC CATCTACCTG GCCCTCCAGG ACTCTGGCCT  
 GGAGGTGAAC ATTGTGACTG CCTCCCAGTA TGCCCTGGGC ATCATCCAGG CCCAGCCTGA  
 TCAGTCTGAG TCTGAGCTGG TGAACCAGAT CATTGAGCAG CTGATCAAGA AGGAGAAGGT  
 5 GTACCTGGCC TGGGTGCCTG CCCACAAGGG CATTGGGGGC AATGAGCAGG TGGACAAGCT  
 GGTGTCTGCT GGCATCAGGA AGGTGCTGTT CCTGGATGGC ATTGACAAGG CCCAGGATGA  
 GCATGAGAAG TACCACTCCA ACTGGAGGGC TATGGCCTCT GACTTCAACC TGCCCCCTGT  
 GGTGGCTAAG GAGATTGTGG CCTCCTGTGA CAAGTGCCAG CTGAAGGGGG AGGCCATGCA  
 TGGGCAGGTG GACTGCTCCC CTGGCATCTG GCAGCTGGCC TGCACCCACC TGGAGGGCAA  
 10 GGTGATCCTG GTGGCTGTGC ATGTGGCCTC CGGCTACATT GAGGCTGAGG TGATCCCTGC  
 TGAGACAGGC CAGGAGACTG CCTACTTCCT GCTGAAGCTG GCTGGCAGGT GGCCTGTGAA  
 GACCATCCAC ACTGCCAATG GCTCCAACCT CACTGGGGCC ACAGTGAGGG CTGCCTGCTG  
 GTGGGCTGGC ATCAAGCAGG AGTTTGGCAT CCCCTACAAC CCCAGTCCC AGGGGGTGGT  
 GGCCTCCATG AACAAGGAGC TGAAGAAGAT CATTGGGCAG GTGAGGGACC AGGCTGAGCA  
 15 CCTGAAGACA GCTGTGCAGA TGGCTGTGTT CATCCACAAC TTCAAGAGGA AGGGGGGCAT  
 CGGGGGCTAC TCCGCTGGGG AGAGGATTGT GGACATCATT GCCACAGACA TCCAGACCAA  
 GGAGCTCCAG AAGCAGATCA CCAAGATCCA GAACTTCAGG GTGTACTACA GGGACTCCAG  
 GAACCCCTG TGGAAGGGCC CTGCCAAGCT GCTGTGGAAG GGGGAGGGGG CTGTGGTGAT  
 CCAGGACAAC TCTGACATCA AGGTGGTGCC CAGGAGGAAG GCCAAGATCA TCAGGGACTA  
 20 TGGCAAGCAG ATGGCTGGGG ATGACTGTGT GGCCTCCAGG CAGGATGAGG ACTAAAGCCC  
 GGGCAGATCT (SEQ ID NO:7).

The open reading frame of the tPA-IA-pol construct disclosed as SEQ ID NO:7 contains 875 amino acids, disclosed herein as tPA-IA-Pol and SEQ ID NO:8, as follows:

25 Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly  
 Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ala Pro Ile Ser Pro Ile  
 Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val  
 Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile  
 Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu  
 30 Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr  
 Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln  
 Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys  
 Lys Lys Lys Ser Val Thr Val Leu Ala Val Gly Asp Ala Tyr Phe Ser  
 Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro  
 35 Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu  
 Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr

Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr  
 Gln Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln  
 His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly  
 Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp  
 5 Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val  
 Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val  
 Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg  
 Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile  
 Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile  
 10 Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu  
 Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile  
 Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met  
 Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln  
 Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe  
 15 Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr  
 Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro  
 Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala  
 Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly  
 Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu  
 20 Thr Asp Thr Thr Asn Gln Lys Thr Ala Leu Gln Ala Ile Tyr Leu Ala  
 Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr  
 Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu  
 Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu  
 Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp  
 25 Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile  
 Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala  
 Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val  
 Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln  
 Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu  
 30 Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu  
 Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu  
 Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Ala Asn  
 Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala  
 Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly  
 35 Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val  
 Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe



Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly  
 Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu  
 Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp  
 Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly  
 5 Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro  
 Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly  
 Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:8).

### EXAMPLE 18

#### 10 CODON OPTIMIZED HIV-1 NEF AND CODON OPTIMIZED HIV-1 NEF MODIFICATIONS

Codon optimized version of HIV-1 Nef and HIV-1 Nef modifications are essentially as described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and PCT International Application PCT/US00/34162, also filed  
 15 December 15, 2000, both documents which are hereby incorporated by reference. As disclosed within the above-mentioned documents, particular embodiments of codon optimized Nef and Nef modifications relate to a DNA molecule encoding HIV-1 Nef from the HIV-1 jf1 isolate wherein the codons are optimized for expression in a mammalian system such as a human. The DNA molecule which encodes this protein  
 20 is disclosed herein as SEQ ID NO:9, while the expressed open reading frame is disclosed herein as SEQ ID NO:10. Another embodiment of Nef-based coding regions for use in the adenoviral vectors of the present invention comprise a codon optimized DNA molecule encoding a protein containing the human plasminogen activator (tpa) leader peptide fused with the NH<sub>2</sub>-terminus of the HIV-1 Nef  
 25 polypeptide. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:11, while the expressed open reading frame is disclosed herein as SEQ ID NO:12. Another modified Nef optimized coding region relates to a DNA molecule encoding optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and  
 30 substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175, herein described as opt nef (G2A, LLAA). The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:13, while the expressed open reading frame is disclosed herein as SEQ ID NO:14. An additional embodiment relates to a DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation  
 35 site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA), comprises an open reading frame which

encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175, herein referred to as opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, while the expressed open reading frame is disclosed herein as SEQ ID NO:16.

5 As disclosed in the above-identified documents (U.S. Application Serial No. 09/738,782 and PCT International Application PCT/US00/34162) and reiterated herein, the following nef-based nucleotide and amino acid sequences which comprise the respective open reading frame are as follows:

1. The nucleotide sequence of the codon optimized version of HIV-1 jfrl  
10 nef gene is disclosed herein as SEQ ID NO:9, as shown herein:

GATCTGCCAC CATGGGCGGC AAGTGGTCCA AGAGGTCCGT GCCCGGCTGG TCCACCGTGA  
GGGAGAGGAT GAGGAGGGCC GAGCCCGCCG CCGACAGGGT GAGGAGGACC GAGCCCGCCG  
CCGTGGGCGT GGGCGCCGTG TCCAGGGACC TGGAGAAGCA CGGCGCCATC ACCTCCTCCA  
ACACCGCCGC CACCAACGCC GACTGCGCCT GGCTGGAGGC CCAGGAGGAC GAGGAGGTGG  
15 GCTTCCCCGT GAGGCCCCAG GTGCCCCCTGA GGCCCATGAC CTACAAGGGC GCCGTGGACC  
TGTCCCACTT CCTGAAGGAG AAGGGCGGCC TGGAGGGCCT GATCCACTCC CAGAAGAGGC  
AGGACATCCT GGACCTGTGG GTGTACCACA CCCAGGGCTA CTTCCCCGAC TGGCAGAACT  
ACACCCCCGG CCCCGGCATC AGGTTCCCCC TGACCTTCGG CTGGTGCTTC AAGCTGGTGC  
CCGTGGAGCC CGAGAAGGTG GAGGAGGCCA ACGAGGGCGA GAACAACTGC CTGCTGCACC  
20 CCATGTCCCA GCACGGCATC GAGGACCCCG AGAAGGAGGT GCTGGAGTGG AGGTTGCACT  
CCAAGCTGGC CTTCCACCAC GTGGCCAGGG AGCTGCACCC CGAGTACTAC AAGGACTGCT  
AAAGCCCCGGG C (SEQ ID NO:9).

Preferred codon usage is as follows: Met (ATG), Gly (GGC), Lys (AAG),  
Trp (TGG), Ser (TCC), Arg (AGG), Val (GTG), Pro (CCC), Thr (ACC), Glu (GAG);  
25 Leu (CTG), His (CAC), Ile (ATC), Asn (AAC), Cys (TGC), Ala (GCC), Gln (CAG),  
Phe (TTC) and Tyr (TAC). For an additional discussion relating to mammalian  
(human) codon optimization, see WO 97/31115 (PCT/US97/02294), which is hereby  
incorporated by reference. See also Figure 19A-B for a comparison of wild type vs.  
codon optimized nucleotides comprising the open reading frame of HIV-Nef.

30 The open reading frame for SEQ ID NO:9 above comprises an initiating  
methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides  
660-662. The open reading frame of SEQ ID NO:9 provides for a 216 amino acid  
HIV-1 Nef protein expressed through utilization of a codon optimized DNA vaccine  
vector. The 216 amino acid HIV-1 Nef (jfrl) protein is disclosed herein as SEQ ID  
35 NO:10, and as follows:

Met Gly Gly Lys Trp Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val

Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg  
 Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu  
 Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp  
 Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val  
 5 Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp  
 Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His  
 Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln  
 Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg  
 Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro  
 10 Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Leu Leu His  
 Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu  
 Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu  
 His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:10).

HIV-1 Nef is a 216 amino acid cytosolic protein which associates with the  
 15 inner surface of the host cell plasma membrane through myristylation of Gly-2  
 (Franchini et al., 1986, *Virology* 155: 593-599). While not all possible Nef functions  
 have been elucidated, it has become clear that correct trafficking of Nef to the inner  
 plasma membrane promotes viral replication by altering the host intracellular  
 environment to facilitate the early phase of the HIV-1 life cycle and by increasing the  
 20 infectivity of progeny viral particles. In one aspect of the invention regarding  
 codon-optimized, protein-modified polypeptides, the nef-encoding region of the  
 adenovirus vector of the present invention is modified to contain a nucleotide  
 sequence which encodes a heterologous leader peptide such that the amino terminal  
 region of the expressed protein will contain the leader peptide. The diversity of  
 25 function that typifies eukaryotic cells depends upon the structural differentiation of  
 their membrane boundaries. To generate and maintain these structures, proteins must  
 be transported from their site of synthesis in the endoplasmic reticulum to  
 predetermined destinations throughout the cell. This requires that the trafficking  
 proteins display sorting signals that are recognized by the molecular machinery  
 30 responsible for route selection located at the access points to the main trafficking  
 pathways. Sorting decisions for most proteins need to be made only once as they  
 traverse their biosynthetic pathways since their final destination, the cellular location  
 at which they perform their function, becomes their permanent residence.  
 Maintenance of intracellular integrity depends in part on the selective sorting and  
 35 accurate transport of proteins to their correct destinations. Defined sequence motifs  
 exist in proteins which can act as 'address labels'. A number of sorting signals have

been found associated with the cytoplasmic domains of membrane proteins. An effective induction of CTL responses often required sustained, high level endogenous expression of an antigen. As membrane-association via myristylation is an essential requirement for most of Nef's function, mutants lacking myristylation, by glycine-to-alanine change, change of the dileucine motif and/or by substitution with a tpa leader sequence as described herein, will be functionally defective, and therefore will have improved safety profile compared to wild-type Nef for use as an HIV-1 vaccine component.

In another embodiment of this portion of the invention, either the DNA vector or the HIV-1 nef nucleotide sequence is modified to include the human tissue-specific plasminogen activator (tPA) leader. As shown in Figure 16A-B, a DNA vector may be modified by known recombinant DNA methodology to contain a leader signal peptide of interest, such that downstream cloning of the modified HIV-1 protein of interest results in a nucleotide sequence which encodes a modified HIV-1 tPA/Nef protein. In the alternative, as noted above, insertion of a nucleotide sequence which encodes a leader peptide may be inserted into a DNA vector housing the open reading frame for the Nef protein of interest. Regardless of the cloning strategy, the end result is a polynucleotide vaccine which comprises vector components for effective gene expression in conjunction with nucleotide sequences which encode a modified HIV-1 Nef protein of interest, including but not limited to a HIV-1 Nef protein which contains a leader peptide. The amino acid sequence of the human tPA leader utilized herein is as follows: MDAMKRGLCCVLLLCGAVFVSPSEISS (SEQ ID NO:17).

It has been shown that myristylation of Gly-2 in conjunction with a dileucine motif in the carboxy region of the protein is essential for Nef-induced down regulation of CD4 (Aiken et al., 1994, *Cell* 76: 853-864) via endocytosis. It has also been shown that Nef expression promotes down regulation of MHCI (Schwartz et al., 1996, *Nature Medicine* 2(3): 338-342) via endocytosis. The present invention relates in part to DNA vaccines which encode modified Nef proteins altered in trafficking and/or functional properties. The modifications introduced into the adenoviral vector HIV vaccines of the present invention include but are not limited to additions, deletions or substitutions to the nef open reading frame which results in the expression of a modified Nef protein which includes an amino terminal leader peptide, modification or deletion of the amino terminal myristylation site, and modification or deletion of the dileucine motif within the Nef protein and which alter function within the infected host cell. Therefore, a central theme of the DNA molecules and recombinant adenoviral HIV vaccines of the present invention is (1)

host administration and intracellular delivery of a codon optimized nef-based adenoviral HIV vaccine; (2) expression of a modified Nef protein which is immunogenic in terms of eliciting both CTL and Th responses; and, (3) inhibiting or at least altering known early viral functions of Nef which have been shown to promote HIV-1 replication and load within an infected host. Therefore, the nef coding region may be altered, resulting in a DNA vaccine which expresses a modified Nef protein wherein the amino terminal Gly-2 myristylation residue is either deleted or modified to express alternate amino acid residues. Also, the nef coding region may be altered so as to result in a DNA vaccine which expresses a modified Nef protein wherein the dileucine motif is either deleted or modified to express alternate amino acid residues. In addition, the adenoviral vector HIV vaccines of the present invention also relate to an isolated DNA molecule, regardless of codon usage, which expresses a wild type or modified Nef protein as described herein, including but not limited to modified Nef proteins which comprise a deletion or substitution of Gly 2, a deletion or substitution of Leu 174 and Leu 175 and/or inclusion of a leader sequence.

Therefore, specific Nef-based constructs further include the following, as exemplification's and not limitations. For example, the present invention relates to an adenoviral vector vaccine which encodes modified forms of HIV-1, an open reading frame which encodes a Nef protein which comprises a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfr1) is referred to herein as opt tpanef. The nucleotide sequence comprising the open reading frame of opt tpanef is disclosed herein as SEQ ID NO:11, as shown below:

```

CATGGATGCA ATGAAGAGAG GGCTCTGCTG TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT
TTCGCCCCAGC GAGATCTCCT CCAAGAGGTC CGTGCCCGGC TGGTCCACCG TGAGGGAGAG
GATGAGGAGG GCCGAGCCCG CCGCCGACAG GGTGAGGAGG ACCGAGCCCG CCGCCGTGGG
CGTGGGCGCC GTGTCCAGGG ACCTGGAGAA GCACGGCGCC ATCACCTCCT CCAACACCGC
CGCCACCAAC GCCGACTGCG CCTGGCTGGA GGCCAGGAG GACGAGGAGG TGGGCTTCCC
CGTGAGGCCC CAGGTGCCCC TGAGGCCCAT GACCTACAAG GGCGCCGTGG ACCTGTCCCA
CTTCCTGAAG GAGAAGGGCG GCCTGGAGGG CTTGATCCAC TCCCAGAAGA GGCAGGACAT
CCTGGACCTG TGGGTGTACC ACACCCAGGG CTACTTCCCC GACTGGCAGA ACTACACCCC
CGGCCCCGGC ATCAGGTTCC CCCTGACCTT CGGCTGGTGC TTCAAGCTGG TGCCCGTGGG
GCCCCAGAAG GTGGAGGAGG CCAACGAGGG CGAGAACAAC TGCCCTGCTGC ACCCATGTG
CCAGCACGGC ATCGAGGACC CCGAGAAGGA GGTGCTGGAG TGGAGGTTCT ACTCCAAGCT
GGCCTTCCAC CACGTGGCCA GGGAGCTGCA CCCCAGTAC TACAAGGACT GCTAAAGCC
(SAQ ID NO:11).

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The open reading frame for SEQ ID NO:11 comprises an initiating methionine

residue at nucleotides 2-4 and a "TAA" stop codon from nucleotides 713-715. The open reading frame of SEQ ID NO:3 provides for a 237 amino acid HIV-1 Nef protein which comprises a tPA leader sequence fused to amino acids 6-216 of HIV-1 Nef, including the dileucine motif at amino acid residues 174 and 175. This 237 amino acid tPA/Nef (jfr1) fusion protein is disclosed herein as SEQ ID NO:12, and is shown as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly  
Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ser Lys Arg Ser Val Pro  
Gly Trp Ser Thr Val Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala  
10 Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val  
Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala  
Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu  
Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr  
Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu  
15 Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp  
Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro  
Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu  
Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn  
Asn Cys Leu Leu His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu  
20 Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His  
Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:12).

Therefore, this exemplified Nef protein, Opt tPA-Nef, contains both a tPA leader sequence as well as deleting the myristylation site of Gly-2A DNA molecule encoding HIV-1 Nef from the HIV-1 jfr1 isolate wherein the codons are optimized for expression in a mammalian system such as a human.

In another specific embodiment of the present invention, a DNA molecule is disclosed which encodes optimized HIV-1 Nef wherein the open reading frame of a recombinant adenoviral HIV vaccine encodes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175. This open reading frame is herein described as opt nef (G2A,LLAA) and is disclosed as SEQ ID NO:13, which comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662. The nucleotide sequence of this codon optimized version of HIV-1 jfr1 nef gene with the above mentioned modifications is disclosed herein as SEQ ID NO:13, as follows:

GATCTGCCAC CATGGCCGGC AAGTGGTCCA AGAGGTCCGT GCCCGGCTGG TCCACCGTGA  
 GGGAGAGGAT GAGGAGGGCC GAGCCCGCCG CCGACAGGGT GAGGAGGACC GAGCCCGCCG  
 CCGTGGGCGT GGGCGCCGTG TCCAGGGACC TGGAGAAGCA CGGCGCCATC ACCTCTCCA  
 ACACCGCCGC CACCAACGCC GACTGCGCCT GGCTGGAGGC CCAGGAGGAC GAGGAGGTGG  
 5 GCTTCCCCGT GAGGCCCCAG GTGCCCCTGA GGCCCATGAC CTACAAGGGC GCCGTGGACC  
 TGTCCCACTT CCTGAAGGAG AAGGGCGGCC TGGAGGGCCT GATCCACTCC CAGAAGAGGC  
 AGGACATCCT GGACCTGTGG GTGTACCACA CCCAGGGCTA CTTCCCCGAC TGGCAGAACT  
 ACACCCCCGG CCCCGGCATC AGGTTCCCCC TGACCTTCGG CTGGTGCTTC AAGCTGGTGC  
 CCGTGGAGCC CGAGAAGGTG GAGGAGGCCA ACGAGGGCGA GAACAACTGC GCCGCCACC  
 10 CCATGTCCA GCACGGCATC GAGGACCCCG AGAAGGAGGT GCTGGAGTGG AGGTTCGACT  
 CCAAGCTGGC CTTCCACCAC GTGGCCAGGG AGCTGCACCC CGAGTACTAC AAGGACTGCT  
 AAAGCCCGGG C (SEQ ID NO:13).

The open reading frame of SEQ ID NO:13 encodes Nef (G2A,LLAA), disclosed herein as SEQ ID NO:14, as follows:

15 Met Ala Gly Lys Trp Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val  
 Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg  
 Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu  
 Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp  
 Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val  
 20 Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp  
 Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His  
 Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln  
 Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg  
 Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro  
 25 Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Ala Ala His  
 Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu  
 Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu  
 His Pro Glu Tyr Tyr Lys Asp Cys Ser (SEQ ID NO:14).

30 An additional embodiment of the present invention relates to another DNA  
 molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation  
 site and dileucine motif have been deleted, as well as comprising a tPA leader peptide.  
 This DNA molecule, opt tpanef (LLAA) comprises an open reading frame which  
 encodes a Nef protein containing a tPA leader sequence fused to amino acid residue  
 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174  
 35 and Ala-175 (Ala-195 and Ala-196 in this tPA-based fusion protein). The nucleotide

sequence comprising the open reading frame of opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, as shown below:

CATGGATGCA ATGAAGAGAG GGCTCTGCTG TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT  
 TTCGCCAGC GAGATCTCCT CCAAGAGGTC CGTGCCCGGC TGGTCCACCG TGAGGGAGAG  
 5 GATGAGGAGG GCCGAGCCCG CCGCCGACAG GGTGAGGAGG ACCGAGCCCG CCGCCGTGGG  
 CGTGGGCGCC GTGTCCAGGG ACCTGGAGAA GCACGGCGCC ATCACCTCCT CCAACACCGC  
 CGCCACCAAC GCCGACTGCG CCTGGCTGGA GGCCAGGAG GACGAGGAGG TGGGCTTCCC  
 CGTGAGGCCC CAGGTGCCCC TGAGGCCCAT GACCTACAAG GGCGCCGTGG ACCTGTCCCA  
 CTTCTGAAG GAGAAGGGCG GCCTGGAGGG CCTGATCCAC TCCCAGAAGA GGCAGGACAT  
 10 CCTGGACCTG TGGGTGTACC ACACCCAGGG CTACTTCCCC GACTGGCAGA ACTACACCCC  
 CGGCCCCGGC ATCAGGTTCC CCCTGACCTT CGGCTGGTGC TTCAAGCTGG TGCCCGTGA  
 GCCCCAGAAG GTGGAGGAGG CCAACGAGGG CGAGAACAAC TGCGCCGCC ACCCCATGTC  
 CCAGCACGGC ATCGAGGACC CCGAGAAGGA GGTGCTGGAG TGGAGGTTCT ACTCCAAGCT  
 GGCCTTCCAC CACGTGGCCA GGGAGCTGCA CCCCAGTAC TACAAGGACT GCTAAAGCCC  
 15 (SEQ ID NO:15).

The open reading frame of SEQ ID NO:7 encoding tPA-Nef (LLAA), disclosed herein as SEQ ID NO:16, is as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly  
 Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ser Lys Arg Ser Val Pro  
 20 Gly Trp Ser Thr Val Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala  
 Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val  
 Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala  
 Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu  
 Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr  
 25 Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu  
 Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp  
 Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro  
 Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu  
 Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn  
 30 Asn Cys Ala Ala His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu  
 Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His  
 Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:16).

An adenoviral vector of the present invention may comprise a DNA sequence,  
 regardless of codon usage, which expresses a wild type or modified Nef protein as  
 35 described herein, including but not limited to modified Nef proteins which comprise a  
 deletion or substitution of Gly 2, a deletion or substitution of Leu 174 and Leu 175



and/or inclusion of a leader sequence. Therefore, partial or fully codon optimized DNA vaccine expression vector constructs are preferred since such constructs should result in increased host expression. However, it is within the scope of the present invention to utilize "non-codon optimized" versions of the constructs disclosed herein, especially modified versions of HIV Nef which are shown to promote a substantial cellular immune response subsequent to host administration.

Figure 20A-C show nucleotide sequences at junctions between nef coding sequence and plasmid backbone of nef expression vectors V1Jns/nef (Figure 20A), V1Jns/nef(G2A,LLAA) (Figure 20B), V1Jns/tpanef (Figure 20C) and V1Jns/tpanef(LLAA) (Figure 20C, also). 5' and 3' flanking sequences of codon optimized nef or codon optimized nef mutant genes are indicated by bold/italic letters; nef and nef mutant coding sequences are indicated by plain letters. Also indicated (as underlined) are the restriction endonuclease sites involved in construction of respective nef expression vectors. V1Jns/tpanef and V1Jns/tpanef(LLAA) have identical sequences at the junctions.

Figure 21 shows a schematic presentation of nef and nef derivatives. Amino acid residues involved in Nef derivatives are presented. Glycine 2 and Leucine 174 and 175 are the sites involved in myristylation and dileucine motif, respectively.

## EXAMPLE 19

### MRKAd5Pol Construction and Virus Rescue

*Construction of vector: shuttle plasmid and pre-adenovirus plasmid - Key steps performed in the construction of the vectors, including the pre-adenovirus plasmid denoted MRKAd5pol, is depicted in Figure 22. Briefly, the adenoviral shuttle vector for the full-length inactivated HIV-1 pol gene is as follows. The vector MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) is a derivative of the shuttle vector used in the construction of the MRKAd5gag adenoviral pre-plasmid. The vector contains an expression cassette with the hCMV promoter (no intronA) and the bovine growth hormone polyadenylation signal. The expression unit has been inserted into the shuttle vector such that insertion of the gene of choice at a unique BglII site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1-/E3+)ClaI (or MRKpAdHVE3) pre-plasmid. The vector, similar to the original shuttle vector contains the PacI site, extension to the packaging signal region, and extension to the pIX gene. The synthetic full-length codon-optimized HIV-1 pol gene was isolated directly from the plasmid pV1Jns-HIV-pol-inact(opt). Digestion of this plasmid with Bgl II releases the pol*

gene intact (comprising a codon optimized IA pol sequence as disclosed in SEQ ID NO:3). The pol fragment was gel purified and ligated into the MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) shuttle vector at the *Bgl*III site. The clones were checked for the correct orientation of the gene by using  
 5 restriction enzymes *Dra*III/*Not*I. A positive clone was isolated and named MRKpdel+hCMVmin+FL-pol+bGHpA(s). The genetic structure of this plasmid was verified by PCR, restriction enzyme and DNA sequencing. The pre-adenovirus plasmid was constructed as follows. Shuttle plasmid MRKpdel+hCMVmin+FL-pol+bGHpA(S) was digested with restriction enzymes *Pac*I and *Bst*1107 I (or its  
 10 isoschizomer, *Bst*Z107 I) and then co-transformed into *E. coli* strain BJ5183 with linearized (*Cla*I digested) adenoviral backbone plasmid, MRKpAd(E1-/E3+)*Cla*I. The resulting pre-plasmid originally named MRKpAd+hCMVmin+FL-pol+bGHpA(S)E3+ is now referred to as "pMRKAd5pol". The genetic structure of the resulting pMRKAd5pol was verified by PCR, restriction enzyme and DNA  
 15 sequence analysis. The vectors were transformed into competent *E. coli* XL-1 Blue for preparative production. The recovered plasmid was verified by restriction enzyme digestion and DNA sequence analysis, and by expression of the pol transgene in transient transfection cell culture. The complete nucleotide sequence of this pMRKAd5HIV-1pol adenoviral vector is shown in Figure 26 A-AO.

20 *Generation of research-grade recombinant adenovirus* - The pre-adenovirus plasmid, pMRKAd5pol, was rescued as infectious virions in PER.C6<sup>®</sup> adherent monolayer cell culture. To rescue infectious virus, 12 µg of pMRKAd5pol was digested with restriction enzyme *Pac*I (New England Biolabs) and 3.3 µg was transfected per 6 cm dish of PER.C6<sup>®</sup> cells using the calcium phosphate co-  
 25 precipitation technique (Cell Pfect Transfection Kit, Amersham Pharmacia Biotech Inc.). *Pac*I digestion releases the viral genome from plasmid sequences allowing viral replication to occur after entry into PER.C6<sup>®</sup> cells. Infected cells and media were harvested 6 -10 days post-transfection, after complete viral cytopathic effect (CPE) was observed. Infected cells and media were stored at ≤ -60°C. This pol containing  
 30 recombinant adenovirus is referred to herein as "MRKAd5pol". This recombinant adenovirus expresses an inactivated HIV-1 Pol protein as shown in SEQ ID NO:6.

## EXAMPLE 20

### MRKAd5Nef Construction and Virus Rescue

35 *Construction of vector: shuttle plasmid and pre-adenovirus plasmid* - Key steps performed in the construction of the vectors, including the pre-adenovirus

plasmid denoted MRKAd5nef, is depicted in Figure 23. Briefly, as shown in Example 19 above, the vector

MRKpdeIE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) is the shuttle vector used in the construction of the MRKAd5gag adenoviral pre-plasmid. It has been modified to contain the *PacI* site, extension to the packaging signal region, and extension to the pIX gene. It contains an expression cassette with the hCMV promoter (no intronA) and the bovine growth hormone polyadenylation signal. The expression unit has been inserted into the shuttle vector such that insertion of the gene of choice at a unique *Bgl*II site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1-/E3+)ClaI pre-plasmid. The synthetic full-length codon-optimized HIV-1 nef gene was isolated directly from the plasmid pV1Jns/nef (G2A,LLAA). Digestion of this plasmid with *Bgl*II releases the pol gene intact, which comprises the nucleotide sequence as disclosed in SEQ ID NO:13. The nef fragment was gel purified and ligated into the MRKpdeIE1+CMVmin+BGHpA(str.) shuttle vector at the *Bgl*II site. The clones were checked for correction orientation of the gene by using restriction enzyme *Sca*I. A positive clone was isolated and named MRKpdeIE1hCMVminFL-nefBGHpA(s). The genetic structure of this plasmid was verified by PCR, restriction enzyme and DNA sequencing. The pre-adenovirus plasmid was constructed as follows. Shuttle plasmid MRKpdeIE1hCMVminFL-nefBGHpA(s) was digested with restriction enzymes *Pac*I and *Bst*1107 I (or its isoschizomer, *Bst*Z107 I) and then co-transformed into *E. coli* strain BJ5183 with linearized (*Cla*I digested) adenoviral backbone plasmid, MRKpAd(E1/E3+)ClaI. The resulting pre-plasmid originally named MRKpdeIE1hCMVminFL-nefBGHpA(s) is now referred to as "pMRKAd5nef". The genetic structure of the resulting pMRKAd5nef was verified by PCR, restriction enzyme and DNA sequence analysis. The vectors were transformed into competent *E. coli* XL-1 Blue for preparative production. The recovered plasmid was verified by restriction enzyme digestion and DNA sequence analysis, and by expression of the nef transgene in transient transfection cell culture. The complete nucleotide sequence of this pMRKAd5HIV-1nef adenoviral vector is shown in Figure 27A-AM.

*Generation of research-grade recombinant adenovirus* - The pre-adenovirus plasmid, pMRKAd5nef, was rescued as infectious virions in PER.C6<sup>®</sup> adherent monolayer cell culture. To rescue infectious virus, 12 µg of pMRKAdnef was digested with restriction enzyme *Pac*I (New England Biolabs) and 3.3 µg was transfected per 6 cm dish of PER.C6<sup>®</sup> cells using the calcium phosphate co-precipitation technique (Cell Pfect Transfection Kit, Amersham Pharmacia Biotech

Inc.). *PacI* digestion releases the viral genome from plasmid sequences allowing viral replication to occur after entry into PER.C6<sup>®</sup> cells. Infected cells and media were harvested 6 -10 days post-transfection, after complete viral cytopathic effect (CPE) was observed. Infected cells and media were stored at  $\leq -60^{\circ}\text{C}$ . This nef containing recombinant adenovirus is now referred to as "MRKAd5nef".

## EXAMPLE 21

### Construction of Murine CMV Promoter Containing Shuttle Vectors for Inactivated Pol and Nef/G2A,LLAA

The murine CMV (mCMV) was amplified from the plasmid pMH4 (supplied by Frank Graham, McMaster University) using the primer set: mCMV (*Not* I) Forward: 5'-ATA AGA ATG CGG CCG CCA TAT ACT GAG TCA TTA GG-3' (SEQ ID NO: 20); mCMV (*Bgl* II) Reverse: 5'-AAG GAA GAT CTA CCG ACG CTG GTC GCG CCT C-3' (SEQ ID NO:21). The underlined nucleotides represent the *Not* I and the *Bgl* II sites respectively for each primer. This PCR amplicon was used for the construction of the mCMV shuttle vector containing the transgene in the E1 parallel orientation. The hCMV promoter was removed from the original shuttle vector (containing the hCMV-gag-bGHpA transgene in the E1 parallel orientation) by digestion with *Not* I and *Bgl* II. The mCMV promoter (*Not* I/*Bgl* II digested PCR product) was inserted into the shuttle vector in a directional manner. The shuttle vector was then digested with *Bgl* II and the gag reporter gene (*Bgl* II fragment) was re-inserted back into the shuttle vector. Several clones were screened for correct orientation of the reporter gene. For the construction of the mCMV-gag in the E1 antiparallel orientation, the mCMV promoter was amplified from the plasmid pMH4 using the following primer set: mCMV (*Asc* I) Forward: 5'- ATA AGA ATG GCG CGC CAT ATA CTG AGT CAT TAG G (SEQ ID NO:22); mCMV (*Bgl* II) Reverse: 5' AAG GAA GAT CTA CCG ACG CTG GTC GCG CCT C (SEQ ID NO:23). The underlined nucleotides represent the *Asc* I and *Bgl* II sites, respectively for each primer. The shuttle vector containing the hCMV-gag transgene in the E1 antiparallel orientation was digested with *Asc* I and *Bgl* II to remove the hCMV-gag portion of the transgene. The mCMV promoter (*Asc* I/*Bgl* II digested PCR product) was inserted into the shuttle vector in a directional manner. The vector was then digested with *Bgl* II and the gag reporter gene (*Bgl* II fragment) was re-inserted. Several clones were screened for correct orientation of the reporter gene. For each of the full length IA pol and full length nef/G2A,LLAA genes, cloning was performed using the unique

*Bgl* II site within the mCMV-bGHpA shuttle vector. The pol and nef genes were excised from their respective pV1Jns plasmids by *Bgl* II digestion.

#### EXAMPLE 22

##### 5 Construction of mCMV Full Length Inactivated Pol and Full Length nef/G2A.LLAA Adenovectors

Each of these transgenes of Example 21 were inserted into the modified shuttle vector in both the E1 parallel and E1 anti-parallel orientations. *Pac*I and *Bst*Z110I digestion of each shuttle vector was performed and each specific transgene  
10 fragment containing the flanking Ad5 sequences was isolated and co-transformed with *Cla* I digested MRKpAd5(E3+) or MRKpAd5(E3-) adenovector plasmids via bacterial homologous recombination in BJ5183 *E. coli* cells. Recombinant pre-plasmid adenovectors containing the various transgenes in both the E3- and E3+ versions (and in the E1 parallel and E1 antiparallel orientations) were subsequently  
15 prepared in large scale following transformation into XL-1 Blue *E. coli* cells and analyzed by restriction analysis and sequencing.

#### EXAMPLE 23

##### Construction of hCMV-tpa-nef (LLAA) Adenovector

20 The tpa-nef gene was amplified out from GMP grade pV1Jns-tpanef (LLAA) vector using the primer sets: Tpanef (BamHI) F 5'-ATT GGA TCC ATG GAT GCA ATG AAG AGA GGG (SEQ ID 24); Tpanef (BamHI) R 5'-ATA GGA TCC TTA GCA GTC CTT GTA GTA CTC G (SEQ ID NO:25). The resulting PCR product was digested with *Bam*HI, gel purified and cloned into the *Bgl* II site of MRKAd5CMV-bGHpA shuttle vector (*Bgl* II digested and calf intestinal phosphatase treated).  
25 Clones containing the tpanef (LLAA) gene (see SEQ ID NO:15 for complet coding region) in the correct orientation with respect to the hCMV promoter were selected following *Sca* I digestion. The resulting MRKAd5tpanef shuttle vector was digested with *Pac* I and *Bst* Z1101 and cloned into the E3+ MRKAd5 adenovector via bacterial  
30 homologous recombination techniques.

#### EXAMPLE 24

##### Immunogenicity of MRKAd5pol and MRKAd5nef Vaccine

Materials and Methods - Rodent Immunization - Groups of N=10 BALB/c  
35 mice were immunized i.m. with the following vectors: (1) MRKAd5hCMV-IApol (E3+) at either  $10^7$  vp and  $10^9$  vp; and (2) MRKAd5hCMV-IApol (E3-) at either

10<sup>7</sup> vp and 10<sup>9</sup> vp. At 7 weeks post dose, 5 of the 10 mice per cohort were boosted with the same vector and dose they initially received. At 3 weeks post the second does, sera and spleens were collected from all the animals for RT ELISA and IFNg ELIspot analyses, respectively. For all rodent immunizations, the Ad5 vectors were  
 5 diluted in 5 mM Tris, 5% sucrose, 75 mM NaCl, 1 mM MgCl<sub>2</sub>, 0.005% polysorbate 80, pH 8.0. The total dose was injected to both quadricep muscles in 50  $\mu$ L aliquots using a 0.3-mL insulin syringe with 28-1/2G needles (Becton-Dickinson, Franklin Lakes, NJ).

Groups of N=10 C57/BL6 mice were immunized i.m. with the following  
 10 vectors: (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+) at either 10<sup>7</sup> vp and 10<sup>9</sup> vp; (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10<sup>7</sup> vp and 10<sup>9</sup> vp; and (3) MRKAd5mCMV-tpanef(LLAA) (E3+) at either 10<sup>7</sup> vp and 10<sup>9</sup> vp. At 7 weeks post dose, 5 of the 10 mice per cohort were boosted with the same vector and dose they initially received. At 3 weeks post the second does, sera and spleens were  
 15 collected from all the animals for RT ELISA and IFNg ELIspot analyses, respectively.

*Non-human Primate immunization* - Cohorts of 3 rhesus macaques (2-3 kg) were vaccinated with the following Ad vectors: (1) MRKAd5hCMV-IApol (E3+) at either 10<sup>9</sup> vp and 10<sup>11</sup> vp dose; and (2) MRKAd5hCMV-IApol (E3-) at either  
 20 10<sup>9</sup> vp and 10<sup>11</sup> vp; (3) MRKAd5hCMV-nef(G2A,LLAA) (E3+) at either 10<sup>9</sup> vp and 10<sup>11</sup> vp; and (4) MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10<sup>9</sup> vp and 10<sup>11</sup> vp. The vaccine was administered to chemically restrained monkeys (10 mg/kg ketamine) by needle injection of two 0.5 mL aliquots of the Ad vectors (in 5 mM Tris, 5% sucrose, 75 mM NaCl, 1 mM MgCl<sub>2</sub>, 0.005% polysorbate 80, pH 8.0)  
 25 into both deltoid muscles. The animals were immunized twice at a 4 week interval (T=0, 4 weeks).

*Murine anti-RT and anti-nef ELISA* - Anti-RT titers were obtained following standard secondary antibody-based ELISA. Maxisorp plates (NUNC, Rochester, NY) were coated by overnight incubation with 100  $\mu$ L of 1  $\mu$ g/mL HIV-1 RT protein  
 30 (Advanced Biotechnologies, Columbia, MD) in PBS. For anti-nef ELISA, 100  $\mu$ L of 1  $\mu$ g/mL HIV-1 nef (Advanced Biotechnologies, Columbia, MD) was used to coat the plates. The plates were washed with PBS/0.05% Tween 20 using Titertek MAP instrument (Hunstville, AL) and incubated for 2 h with 200  $\mu$ L/well of blocking solution (PBS/0.05% tween/1% BSA). An initial serum dilution of 100-fold was  
 35 performed followed by 4-fold serial dilution. 100- $\mu$ L aliquots of serially diluted samples were added per well and incubated for 2 h at room temperature. The plates

were washed and 100  $\mu$ L of 1/1000-diluted HRP-rabbit anti-mouse IgG (ZYMED, San Francisco, CA) were added with 1 h incubation. The plates were washed thoroughly and soaked with 100  $\mu$ L 1,2-phenylenediamine dihydrochloride/hydrogen peroxide (DAKO, Norway) solution for 15 min. The reaction was quenched by adding 100  $\mu$ L of 0.5M  $H_2SO_4$  per well. OD<sub>492</sub> readings were recorded using Titertek Multiskan MCC/340 with S20 stacker. Endpoint titers were defined as the highest serum dilution that resulted in an absorbance value of greater than or equal to 0.1 OD<sub>492</sub> (2.5 times the background value).

*Non-human primate and murine ELISpot assays* - The enzyme-linked immuno-spot (ELISpot) assay was utilized to enumerate antigen-specific INF $\gamma$ -secreting cells from mouse spleens (Miyahira, et al.1995, *J. Immunol. Methods* 181:45-54) or macaque PBMCs. Mouse spleens were pooled from 5 mice/cohort and single cell suspensions were prepared at  $5 \times 10^6$ /mL in complete RPMI media (RPMI1640, 10% FBS, 2mM L-glutamine, 100U/mL Penicillin, 100 u/mL streptomycin, 10 mM Hepes, 50 uM  $\beta$ -ME). Rhesus PBMCs were prepared from 8-15 mL of heparinized blood following standard Ficoll gradient separation (Coligan, et al, 1998, *Current Protocols in Immunology*. John Wiley & Sons, Inc.). Multiscreen opaque plates (Millipore, France) were coated with 100  $\mu$ L/well of either 5  $\mu$ g/mL purified rat anti-mouse IFN- $\gamma$  IgG1, clone R4-6A2 (Pharmingen, San Diego, CA), or 15  $\mu$ g/mL mouse anti-human IFN- $\gamma$  IgG<sub>2a</sub> (Cat. No. 1598-00, R&D Systems, Minneapolis, MN) in PBS at 4°C overnight for murine or monkey assays, respectively. The plates were washed with PBS/penicillin/streptomycin and blocked with 200  $\mu$ L/well of complete RPMI media for 37 °C for at least 2 h.

To each well, 50  $\mu$ L of cell samples ( $4-5 \times 10^5$  cells per well) and 50  $\mu$ L of the antigen solution were added. To the control well, 50  $\mu$ L of the media containing DMSO were added; for specific responses, either selected peptides or peptide pools (4  $\mu$ g/mL per peptide final concentration) were added. For BALB/c mice immunized with the pol constructs, stimulation was conducted using a pool of CD4<sup>+</sup>-epitope containing 20-mer peptides (aa21-40, aa411-430, aa641-660, aa731-750, aa771-790) or a pool of CD8<sup>+</sup>-epitope containing peptides (aa201-220, aa311-330, aa781-800). For C57/BL6 mice immunized with the nef construct, either aa51-70 (CD8<sup>+</sup> T cell epitope) or aa81-100 (CD4<sup>+</sup>) peptide derived from the nef sequence was added for specific stimulation. In monkeys, the responses against pol were evaluated using two pools (L and R) of 20-aa peptides that encompass the entire pol sequence and overlap by 10 amino acids. In monkeys vaccinated with the nef constructs, a single pool containing 20-mer peptides covering the entire HIV-1 nef sequence and overlapping

by 10 aa was used. Each sample/antigen mixture was performed in triplicate wells for murine samples or in duplicate wells for rhesus PBMCs. Plates were incubated at 37°C, 5% CO<sub>2</sub>, 90% humidity for 20-24 h. The plates were washed with PBS/0.05% Tween 20 and incubated with 100 µL/well of either 1.25 µg/mL biotin-conjugated rat anti-mouse IFN-γ mAb, clone XMG1.2 (Pharmingen) or of 0.1 µg/mL biotinylated anti-human IFN-gamma goat polyclonal antibody (R&D Systems) at 4°C overnight. The plates were washed and incubated with 100 µL/well 1/2500 dilution of streptavidin-alkaline phosphatase conjugate (Pharmingen) in PBS/0.005% Tween/5% FBS for 30 min at 37 °C. Spots were developed by incubating with 100 µL/well 1-step NBT/BCIP (Pierce Chemicals) for 6-10 min. The plates were washed with water and allowed to air dry. The number of spots in each well was determined using a dissecting microscope and the data normalized to 10<sup>6</sup> cell input.

*Non-human Primate anti-RT ELISA* - The pol-specific antibodies in the monkeys were measured in a competitive RT EIA assay, wherein sample activity is determined by the ability to block RT antigen from binding to coating antibody on the plate well. Briefly, Maxisorp plates were coated with saturating amounts of pol positive human serum (#97111234). 250 µL of each sample is incubated with 15 µL of 266 ng/mL RT recombinant protein (in RCM 563, 1% BSA, 0.1% tween, 0.1% NaN<sub>3</sub>) and 20 µL of lysis buffer (Coulter p24 antigen assay kit) for 15 min at room temperature. Similar mixtures are prepared using serially diluted samples of a standard and a negative control which defines maximum RT binding. 200 µL/well of each sample and standard were added to the washed plate and the plate incubated 16-24 h at room temperature. Bound RT is quantified following the procedures described in Coulter p24 assay kit and reported in milliMerck units per mL arbitrarily defined by the chosen standard.

*Results - Rodent Studies* - BALB/c mice (n=5 mice/cohort) were immunized once or twice with varying doses of MRKAd5hCMV-IAPol(E3+) and MRKAd5hCMV-IAPol(E3-). At 3 weeks after the second dose, Anti-pol IgG levels were determined by an ELISA assay using RT as a surrogate antigen. Cellular response were quantified via IFNγ ELISpot assay against pools of pol-epitope containing peptides. The results of these assays are summarized in Table 10. The results indicate that the mouse vaccinees exhibited detectable anti-RT IgGs with an adenovector dose as low as 10<sup>7</sup> vp. The humoral responses are highly dose-dependent and are boostable with a second immunization. One or two doses of either pol vectors elicit high frequencies of antigen-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells; the responses are weakly dose-dependent but are boostable with a second immunization.



Table 10. Immunogenicity of MRKAd5pol Vectors in BALB/c mice.

Group	Vaccine	Dose	No. of Doses	Anti-RT IgG Titers <sup>a</sup>			SFC/10 <sup>6</sup> cells <sup>b</sup>		
				GMT	+SE	-SE	Medium	CD4+ peptide pool	CD8+ peptide pool
1	MRKAd5hCMVFLpol (E3+)	10 <sup>7</sup> vp	2 1	310419 919	301785 372	153020 265	1(1) 1(1)	75(4) 72(9)	2313(87) 533(41)
2	MRKAd5hCMVFLpol (E3+)	10 <sup>9</sup> vp	2 1	1838400 <sup>b</sup> 713155	0 528520	0 303555	2(2) 1(1)	114(9) 48(7)	2083(182) 733(89)
3	MRKAd5hCMVFLpol (E3-)	10 <sup>7</sup> vp	2 1	310419 6400	385218 14013	172097 4393	0(0) 10(8)	223(7) 141(21)	2807(27) 409(28)
4	MRKAd5hCMVFLpol (E3-)	10 <sup>9</sup> vp	2 1	1838400 <sup>b</sup> 1241675 <sup>b</sup>	0 396725	0 300661	1(1) 0(0)	160(13) 39(13)	2385(11) 833(83)
5	Naïve	none	none	57	9	7	9(2)	11(4)	10(1)

<sup>a</sup>GMT, geometric mean titer of the cohort of 5 mice; SE, standard error of the geometric mean<sup>b</sup>Near or at the upper limit of the serial dilution; hence, could be greater than this value<sup>c</sup>No. of Spot-forming Cells per million splenocytes; mean values of triplicates are reported along with standard errors in parenthesis.

- 5 C57/BL6 mice were immunized once or twice with varying doses of MRKAd5hCMV-nef(G2A,LLAA) (E3+), MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10<sup>7</sup> vp and (3) MRKAd5mCMV-tpanef(LLAA) (E3+) at either 10<sup>7</sup> vp and 10<sup>9</sup> vp. The immune response were analyzed using similar protocols and the results are listed in Table 11. While anti-nef IgG responses could not be detected in this
- 10 model system with any of the constructs, there are strong indications of a cellular immunity generated against nef using the ELIspot assay.

Table 11. Immunogenicity of MRKAd5nef Vectors in C57/BL6 mice.

Group	Vaccine	Dose	No. of Doses	Anti-nef IgG Titers <sup>a</sup>			SFC/10 <sup>6</sup> cells <sup>b</sup>		
				GMT	+SE	-SE	Medium	aa51-70 CD8+	aa81-100 CD4+
1	MRKAd5hCMVFLnef (E3+)	10 <sup>7</sup> vp	2 1	174 132	70 42	50 32	1(1) 0(0)	23(1) 0(0)	1(1) 0(0)
2	MRKAd5hCMVFLnef (E3+)	10 <sup>9</sup> vp	2 1	174 132	70 42	50 32	0(0) 1(1)	61(7) 62(7)	4(2) 3(1)
3	MRKAd5mCMVFLnef (E3+)	10 <sup>7</sup> vp	2 1	132 115	42 46	32 33	3(1) 3(2)	15(5) 3(2)	5(2) 4(2)
4	MRKAd5mCMVFLnef (E3+)	10 <sup>9</sup> vp	2 1	132 132	42 42	32 32	4(2) 2(1)	83(13) 29(2)	5(1) 4(0)
5	MRKAd5mCMVtpanef(E3+)	10 <sup>7</sup> vp	2 1	132 100	42 0	32 0	3(2) 3(1)	14(2) 13(4)	5(1) 10(3)
6	MRKAd5mCMVtpanef(E3+)	10 <sup>9</sup> vp	2 1	230 115	170 46	98 33	3(2) 7(1)	145(29) 151(14)	4(0) 10(0)
7	Naïve	none	none	152	78	52	21(2)	18(6)	28(3)

<sup>a</sup>GMT, geometric mean titer of the cohort of 5 mice; SE, standard error of the geometric mean<sup>b</sup>No. of spot-forming cells per million splenocytes; mean values of triplicates are reported along with standard errors in parenthesis.

15

*Monkey Studies* - Cohorts of 3 rhesus macaques were immunized with 2 doses of MRKAd5hCMV-IAPol(E3+) and MRKAd5hCMV-IAPol(E3-). The number of antigen-specific T cells (per million PBMCs) were enumerated using one of two

- peptide pools (L and R) that cover the entire pol sequence; the results are listed in Table 12. Moderate-to-strong T cell responses were detected in the vaccinees using either constructs even at a low dose of  $10^9$  vp. Longitudinal analyses of the anti-RT antibody titers in the animals suggest that the pol transgene product is expressed efficiently to elicit a humoral response (Table 13). It would appear that generally higher immune responses were observed in animals that received the E3- construct compared to the E3+ virus.

Table 12. Pol-specific T Cell Responses in MRKAd5pol Immunized Rhesus Macaques.

Vaccine (T=0,4 wks)	Monkey #	Prebleed			T=4			T=7			T=16		
		Mock	Pol L	Pol R	Mock	Pol L	Pol R	Mock	Pol L	Pol R	Mock	Pol L	Pol R
MRKAd5hCMV-IAPol(E3+), $10^{11}$ vp	99C100	1	0	0	1	38	31	0	52	148	0	49	715
	99C215	1	2	2	10	98	249	1	109	305	22	88	250
	99D201	5	5	4	6	149	85	0	40	35	0	35	18
MRKAd5hCMV-IAPol(E3+), $10^9$ vp	99D212	0	2	0	4	331	114	0	58	14	0	6	6
	99D180	0	4	2	0	19	182	4	38	156	5	38	108
	99C201	8	5	21	6	62	62	0	18	32	1	14	65
MRKAd5hCMV-IAPol(E3-), $10^{11}$ vp	99D239	5	2	2	20	82	172	1	68	114	9	21	40
	99C186	4	12	8	5	120	421	2	271	489	16	875	530
	99C084	1	8	9	8	84	484	0	14	238	1	24	284
MRKAd5hCMV-IAPol(E3-), $10^9$ vp	CC7C	10	10	8	12	724	745	4	322	376	4	188	178
	CD1G	2	0	1	5	474	468	0	232	212	0	101	121
	CD11	8	6	12	10	98	110	5	60	80	8	25	34
Native	083Q	nd	nd	nd	nd	nd	nd	4	2	2	2	1	2

nd, not determined  
Reported are SFC per million PBMCs; mean of duplicate wells.

Table 13. Anti-RT Ig Levels in MRKAd5pol Immunized macaques.

RT ANTIBODY ASSAY TITERS IN mMU/ml				
Vaccine/Monkey Tag	T=4	T=7	T=12	T=16
MRKAd5hCMV-IAPol(E3+), $10^{11}$ vp				
99C100	61	1999	5928	4768
99C215	81	1541	2356	2767
99D201	53	336	539	387
MRKAd5hCMV-IAPol(E3+), $10^9$ vp				
99D212	10	40	49	68
99D180	<10	36	79	93
99C201	<10	37	71	76
MRKAd5hCMV-IAPol(E3-), $10^{11}$ vp				
99D239	44	460	1234	1015
99C186	21	233	480	345
99C084	235	2637	2858	1626
MRKAd5hCMV-IAPol(E3-), $10^9$ vp				
CC7C	32	175	306	235
CD1G	20	140	273	419
CD11	15	112	149	237

When rhesus macaques were immunized i.m. with two doses of MRKAd5nef

- 5 constructs, vigorous T cell responses ranging from 100 to as high as 1100 per million were observed in 8 of 12 vaccinees (Table 14). The efficacies of the mCMV- and hCMV- driven nef constructs are comparable on the basis of the data generated thus far.

10 Table 14. Nef-specific T cell Responses in MRKAd5nef Immunized Rhesus Macaques.

Vaccine (T=0,4 wks)	Monk #	Pre		T=4		T=7		T=16	
		Mock	Nef	Mock	Nef	Mock	Nef	Mock	Nef
MRKAd5hCMV-nef(G2A,LLAA) (E3+) 10 <sup>11</sup> vp	CD2D	0	4	31	440	4	368	1	251
	CC7B	0	0	2	521	0	178	1	1522
	CC61	2	9	31	112	0	108	11	100
MRKAd5hCMV-nef(G2A,LLAA) (E3+) 10 <sup>9</sup> vp	CC2K	9	9	6	52	0	35	0	15
	CD15	5	4	30	898	2	588	0	434
	CD16	6	1	8	1148	0	369	1	212
MRKAd5mCMV-nef(G2A,LLAA) (E3+) 10 <sup>11</sup> vp	99D191	1	5	4	614	0	298	2	419
	99D144	4	6	5	434	0	1100	2	932
	99C193	1	2	1	58	1	22	0	64
MRKAd5mCMV-nef(G2A,LLAA) (E3+) 10 <sup>9</sup> vp	99D224	1	11	14	231	1	125	0	70
	99D250	8	9	4	108	0	54	0	5
	99C120	1	6	20	299	0	92	0	79
Naïve	083Q	nd	nd	18	22	4	5	2	1

#### EXAMPLE 25

- 15 Comparison of Clade B vs. Clade C T Cell Responses in HIV-Infected Subjects

PBMC samples collected from two dozens of patients infected with HIV-1 in US were tested in ELISPOT assays with peptide pools of 20-mer peptides overlapping by 10 amino acids. Four different peptide pools were tested for cross-clade recognition, and they were either derived from a clade B-based isolate (gag H-b; nef-  
 20 b) or a clade C-based isolate (gag H-c, nef-c). Data in Table 15 shows that T cells from these patients presumably infected with clade B HIV-1 could recognize clade C gag and nef antigens in ELISPOT assay. Correlation analysis further demonstrated that these T cell responses against clade C gag peptide pool were about 60% of the clade B counterpart (Figure 24), while the T cell responses against clade C nef were  
 25 about 85% of the clade B counterpart (Figure 25). These results suggest that cellular immune responses generated in patients infected with clade B HIV-1 can recognize gag and nef antigens derived from clade C HIV-1. These data show that a HIV vaccine, such as a DNA or MRKAd5-based adenoviral vaccine expressing a clade B

gag and/or nef antigen will potentially have the ability to provide a prophylactic and/or therapeutic advantage on a global scale.

5

Table 15  
Responses Shown as the Number of gIFN-Secreting T Cells per Million PBMCs

subject	bleed date	gag epitope # (from mapping)	mock	gag H-b	gagH-c	nef-b	nef-c
#100	19-Jul-99	12	10	3950	1385	1295	1300
#101	25-Jul-99	3	15	3885	1280	na	1020
#102	25-Jul-99	4	15	1740	850	1255	1785
#104	7-Jun-99	2	5	1355	1185	na	1060
#107	11-Oct-99	2	25	3305	2795	670	870
#405	11-Jul-99	2	15	4575	3180	1700	1500
#501	19-Jul-99	2	15	1100	570	3365	3460
#505	18-Jul-99	5	10	2145	1725	1235	na
#506	28-Feb-99	2	25	150	45	400	610
#701	28-Mar-99	5	30	7620	4775	3320	2780
#709	17-May-99	3	15	2785	1945	1090	1630
#710	24-May-99	4	5	1055	1080	2210	2140

10

#### EXAMPLE 26

#### Characterization and Production of MRKAd5pol and MRKAd5nef Vectors in Roller Bottles

*Expansion of nef and pol Adenovectors* - Nef and pol CsCl purified MRKAd5 seeds were used to infect roller bottles to produce P4 virus to be used as a seed for further experiments. P4 MRKAd5 pol and nef vectors were used to infect roller bottles at an MOI 280 vp/cell, except for hCMV-tpa-nef [E3+] which was infected at an MOI of 125 due to low titers of seed obtained at P4.

20

Table 16 Viral particle concentrations for P5 nef and pol adenovectors

Adenovector	AEX Titer (10 <sup>10</sup> vp/ml culture)	AEX Titer (10 <sup>4</sup> vp/cell)	Amplification Ratio
hCMV-FL-nef [E3+]	1.1	0.9	30
mCMV-FL-nef [E3+]	2.2	2.1	75
hCMV-tpa-nef [E3+]	0.07	0.1	5
mCMV-tpa-nef [E3+]	1.3	0.9	35
hCMV-FL-pol [E3+]	2.7	2.1	75
hCMV-FL-pol [E3-]	1.9	1.3	45

- 5 *Roller Bottle Passaging* - Passaging of the *pol* and *nef* constructs continued through passage seven. Cell-associated (freeze/thaw lysis) and whole broth (triton-lysis) titers obtained in all passages were very consistent. In general, MRKAd5pol is ca. 70% as productive as MRKAd5gag while MRKAd5nef is ca. 25% as productive as MRKAd5gag. Samples of P7 virus for both constructs were analyzed by V&CB by
- 10 restriction digest analysis and did not show any rearrangements.

Table 17. Passage Six Viral Productivity for MRKAd5pol and MRKAd5nef

		Xviable (10 <sup>6</sup> cells/ml), Viability (%)		Cell Passage Number	AEX Titer (Cell Associated) 10 <sup>10</sup> vp/ml culture	Titer 10 <sup>4</sup> vp/cell	Amplification Ratio	Triton Lysis Titer 10 <sup>10</sup> vp/ml culture
		Infection	Harvest					
hCMV-FL-nef [E3+]	pool	1.22, 85%		62	0.8	0.7	25	1.6
	1		0.99, 62%					
	2		1.10, 72%					
hCMV-FL-pol [E3+]	pool	1.42, 89%		62	4.5	3.2	115	7.0
	1		1.22, 70%					
	2		1.42, 74%					

15 Table 18. Passage Seven Viral Productivity for MRKAd5pol and MRKAd5nef

		Xviable (10 <sup>6</sup> cells/ml), Viability (%)		Cell Passage Number	AEX Titer (Cell Associated) 10 <sup>10</sup> vp/ml culture	Titer 10 <sup>4</sup> vp/cell	Amplification Ratio	Triton Lysis Titer 10 <sup>10</sup> vp/ml culture
		Infection	Harvest					
hCMV-FL-nef [E3+]	Pool	1.33, 90%		66	1.0	0.8	29	2.1
	1		0.96, 70%					
	2		1.18, 73%					
hCMV-FL-pol [E3+]	Pool	0.90*, 90%		56	4.2	4.7	168	6.5
	1		1.18, 88%					
	2		1.04, 80%					

- MRKAd5nef and MRKAd5pol Viral Production Kinetics* - A timecourse experiment was carried out in roller bottles to determine if the viral production kinetics of the MRKAd5pol and MRKAd5nef vectors were similar to those of MRKAd5gag. PER.C6<sup>®</sup> cells in roller bottle cultures were infected at an MOI of 280 vp/cells with P5 MRKAd5pol, P5 MRKAd5nef and P7 MRKAd5gag; for each adenovector, two infected bottles were sampled at 24, 36, 48, and 60 hours post infection. In addition, two bottles were left unsampled until 48 hpi when they were harvested under the Phase I process conditions. The anion-exchange HPLC viral
- 20 particle concentrations of the freeze-thaw recovered cell associated virus at the 24, 36,
- 25

48, and 60 hpi timepoints are shown in Figure 29A-B. The QPA titers show a similar trend (data not shown).

- Comparison of hCMV- and mCMV-FL-nef* - As the titers obtained with the MRKAd5nef construct (hCMV-FL-nef) were lower than those obtained with MRKAd5gag or MRKAd5pol, a viral productivity comparison experiment was performed with mCMV-FL-nef. For each of the two adenovectors (hCMV- and mCMV-FL-nef), two roller bottles were infected at an MOI of 280 vp/cell with passage five clarified lysate. The macroscopic and microscopic observations of the four roller bottles were identical at the time of harvest. Analysis of the clarified lysate produced indicated a higher viral particle concentration in the bottles infected with mCMV-FL-nef, as shown in Table 19. It is stipulated that the higher productivity with mCMV promoter driven nef vector is due to lower nef expression levels in PER.C6® cells- experiments are underway at V&CB to measure nef expression levels.

Table 19. Passage Six Viral Productivity Comparison of hCMV- and mCMV-FL-nef

		Xv (10 <sup>6</sup> cells/ml), Viability (%)		Cell Passage	ABX Titer	Titer	Amplification	Triton Lysis Titer
		Infection	Harvest	Number	10 <sup>10</sup> vp/ml culture	10 <sup>4</sup> vp/cell	Ratio	10 <sup>10</sup> vp/ml culture
hCMV-FL-nef (MRKAd5nef)	Pool	1.11, 91%		60	1.5	1.4	50	2.8
	1		1.23, 75%					
	2		1.34, 74%					
mCMV-FL-nef	Pool	1.11, 91%		60	2.3	2.1	75	4.6
	1		1.49, 84%					
	2		1.18, 77%					

20

### EXAMPLE 27

#### Characterization and Large Scale Production of MRKAd5nef Virus in Bioreactors

- Materials and Methods* - The experiment of the present example was run twice under the following conditions: 36.5°C, DO 30%, pH 7.30, 150rpm agitation rate, no sparging, Life Technologies (Gibco, Invitrogen) 293 SFM II (with 6mM L-glutamine), 0.5M NaOH as base for pH control. During the first run (B20010115), two 10L stirred vessel bioreactors were inoculated with PER.C6® cells at a concentration of 0.2x10<sup>6</sup> cells/ml. Cells were grown until they reached a cell concentration of approximately 1x10<sup>6</sup> cells/ml. The cells were infected with uncloned MRKAd5nef (G2A,LLAA) at a MOI of 280 virus particles (vp)/cell. For the second batch (B20010202), the same procedure as the first run was used, except the cells

were infected with cloned MRAd5nef. During both runs, the bioreactors were harvested 48 hours post-infection. Samples were taken and virus concentrations were determined from whole broth (with triton lysis), supernatant, and cell pellets (3 X freeze/thaw) with the AEX and QPA assays. Metabolites were measured with BioProfile 250 throughout the process.

Table 20: Experimental Conditions

Temperature	36.5 °C
DO	30%
PH	7.30
Agitation	150 rpm
Sparging	None

Table 21: Virus source used for experiments.

Run	Batch ID	Cloned/Uncloned MRKAd5nef	MOI (vp/cells)
#1	B20010115-1	Uncloned	280
	B20010115-2	Uncloned	280
#2	B20010202-1	Cloned	280
	B20010202-2	Cloned	280

*Results* - Table 22 and 23 show an the ability to scale up production of MRKAd5nef by growth in a bioreactor.

Table 22: Virus Concentration as measured by the AEX assay

Run	Batch ID	Cloned/Uncloned MRKAd5nef	Virus Concentration @ 48hpi (1x10 <sup>13</sup> vp/L)			
			Supernatant	Clarified Lysate	Total	Triton Lysate
#1	B20010115-1	Uncloned	0.72	3.26	3.98	5.76
	B20010115-2	Uncloned	0.38	1.67	2.05	2.46
#2	B20010202-1	Cloned	0.80	6.00	6.80	8.88
	B20010202-2	Cloned	0.50	6.00	6.50	8.47

Table 23: Virus Titers as measured by the QPA assay

Run	Batch ID	Cloned/Uncloned MRKAd5nef	Virus Concentration @ 48hpi (1x10 <sup>11</sup> IU/L)				
			Whole Broth	Supernatant	Clarified Lysate	Total	Triton Lysate
#1	B20010115-1	Uncloned	0.13	1.12	1.76	2.88	11.28
	B20010115-2	Uncloned	0.14	0.73	1.54	2.27	5.86
#2	B20010202-1	Cloned	0.14	0.97	1.62	2.69	11.89
	B20010202-2	Cloned	0.14	1.17	1.70	2.97	12.47

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art

from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

#### EXAMPLE 28

##### 5 MRKAd5HIV-1gag Boosting of DNA-Primed Animals

Groups of 3-5 rhesus macaques were immunized with (a) 5 mgs of V1Jns-Flgag (pV1JnsCMV(no intron)-FL-gag-bGHpA), (b) 5 mgs of V1Jns-Flgag formulated with 45 mgs of a non-ionic block copolymer CRL1005, or (c) 5 mgs of V1Jns-Flgag formulated with 7.5 mgs of CRL1005 and 0.6 mM benzalkonium chloride at weeks 0, 4, and 8. All animals received a single dose of  $10^7$  viral particles (vp) of the MRKAd5HIV-1gag at week 26. Note:  $10^7$  is too low to prime or boost effectively when used as a single modality (dose is selected to mimic preexposure to adenovirus); see Figure 32.

15 Blood samples were collected from all animals at several time points and peripheral blood mononuclear cells (PBMCs) were prepared using standard Ficoll method. The PBMCs were counted and analyzed for gamma-interferon secretion using the ELISpot assay (Table 24). For each monkey, the PBMCs were incubated overnight either in the absence (medium) or presence of a pool (called "gag H") of 50 20-aa long peptides that encompass the entire HIV-1 gag sequence.

The results indicate that MRKAd5HIV-1gag was very effective in boosting the T cell immune responses in these monkeys. At week 28 or 2 weeks after the viral boost, the number of gag-specific T cells per million PBMCs increased 2-48 fold compared to the levels observed at week 24 or 2 weeks prior to the boost.

25 The PBMCs were also analyzed by intracellular gamma-interferon staining prior to (at week 10) and after the MRKAd5gag boost (at week 30). The results for select animals are shown on Figure 31. The results indicate that (a) immunization with DNA/adjuvant formulation elicited T cell responses which can either be balanced,  $CD4^+$ -biased or  $CD8^+$ -biased, and (b) boosting with the MRKAd5gag 30 construct produced in all cases a strongly  $CD8^+$ -biased response. These results suggest that boosting with MRKAd5HIV-1gag construct is able to improve the levels of antigen-specific  $CD8^+$  T cells.



Table 24. Boosting of DNA/Adjuvant-Primed Rhesus Monkeys with MRKA5 gag

Group	Priming	Boost	Monkey	T=0		T=4		T=6		T=10		T=17		T=24		T=28		T=30	
				Medium	gag H	Medium	gag H	Medium	gag H	Medium	gag H	Medium	gag H	Medium	gag H	Medium	gag H	Medium	gag H
1	T=0, 4, 8 wks DNA/5 mgs PBS (D101)	T=28 wks MRKA5 gag(E3+) 10 <sup>7</sup> vp	CB5H CB5K AW3G	NA	NA	3	35	15	71	4	224	8	116	6	85	19	956	0	316
				0	0	0	15	0	48	0	68	0	76	0	35	3	1705	1	755
				5	11	0	36	3	51	3	48	2	69	8	65	10	889	0	395
2	DNA/5mgs + CRL1005/5mgs	MRKA5 gag(E3+) 10 <sup>7</sup> vp	CC1C CC1K AW3P CB5F AW3B	0	4	1	60	0	111	5	270	4	280	8	232	3	939	19	1345
				4	0	1	101	0	254	0	781	5	482	0	321	0	1915	1	1099
				9	8	1	10	4	71	4	154	8	104	5	85	11	636	6	241
				NA	NA	0	31	0	288	0	530	19	374	9	251	8	1549	20	1734
				8	12	4	36	1	119	0	439	0	425	0	316	4	1229	5	1354
3	DNA/5 mgs + CRL1005/7.5 mgs + 0.8 mM BAK	MRKA5 gag(E3+) 10 <sup>7</sup> vp	AW20 CA4R CB5B CB5W CB7D	10	4	1	59	5	264	19	425	8	105	9	203	18	563	8	404
				1	0	3	121	1	135	1	270	5	130	1	105	14	1384	10	978
				8	6	0	8	3	119	0	274	8	282	1	209	0	638	1	828
				4	3	0	26	1	91	0	139	0	164	1	62	5	543	1	348
				1	0	0	136	0	316	1	609	5	826	1	769	0	2278	4	1831
4	none	None	88D201	3	0	0	0	1	0	0	0	0	1	1	2	3	0	0	0

NA, not available

## EXAMPLE 29

## Construction of gagpol fusion for MRKAd5gagpol fusion constructs

5 The open reading frames for the codon-optimized HIV-1 gag gene was fused directly to the open reading frame of the IA pol gene (consisting of RT, RNaseH and integrase domains) by stepwise PCR. Because the gene (SEQ ID NO: 38) does not include the protease gene and the frameshift sequence, it encodes a single polypeptide of the combined size of p55, RT, RNase H and integrase (1350 amino acids; SEQ ID NO: 39).

10 The fragment that extends from the BstEII site within the gag gene to the last non-stop codon was ligated via PCR to a fragment that extends from the start codon of the IApol to a unique BamHI site. This fragment was digested with BstEII and BamHI. Construction of gag-IApol fusion was achieved via three-fragment ligation involving the PstI-BstEII gag digestion fragment, the BstEII/BamHI digested PCR  
15 product and long PstI/BamHI V1R-FLpol backbone fragment.

The MRKAd5-gagpol adenovirus vector was constructed using the BglII fragment of the V1R-gagpol containing the entire ORF of gag-IApol fusion gene.

## EXAMPLE 30

## 20 Immunogenicity Studies in Non-Human Primates

Cohorts of three (3) macaques were immunized with 10e8 or 10e10 viral particles (vp) of one of the following MRKAd5 HIV-1 vaccines: (1) MRKAd5gag; (2) MRKAd5pol; (3) MRKAd5nef; (4) a mixture containing equal amounts of  
25 MRKAd5gag, MRKAd5pol, and MRKAd5nef, or (5) a mixture of equal amounts of MRKAd5gagpol and MRKAd5nef. The vaccines were administered at weeks 0 and 4.

The T cell responses against each of the HIV-1 antigens were assayed by IFN-gamma ELISpot assay using pools of 20-aa peptides that encompass the entire protein sequence of each antigen. The results (Table 25) are expressed as the number of spot-forming cells (sfc) per million peripheral blood mononuclear cells (PBMC) that  
30 respond to each of the peptide pools.

Results indicate the following observations: (1) each of the single gene constructs (MRKAd5gag, MRKAd5pol, or MRKAd5nef) is able to elicit high levels  
35 of antigen-specific T cells in monkeys; (2) the single-gene MRKAd5 constructs can be mixed as a multi-cocktail formulation capable of eliciting very broad T cell responses against gag, pol, and nef; (3) the MRKAd5 vector expressing the fusion

protein of gag plus IA pol is capable of inducing strong T cell responses to both gag and pol.

**Table 25. Evaluation of Mixtures of MRKAd5 vectors expressing humanized HIV-1 gag, pol, gagpol, nef in rhesus macaques**

Grp #	Vaccine T=0, 4 wks	Monk #	T=6 wks				
			Mock	Gag H	Pol - 1	Pol - 2	Nef
1	MRKAd5 gag 10 <sup>10</sup> vp	CB9V	0	15	-	-	-
		CD19	0	374	-	-	-
		109H	1	843	-	-	-
2	MRKAd5 gag 10 <sup>8</sup> vp	99D130	1	948	-	-	-
		W277	16	324	-	-	-
		143H	4	595	-	-	-
3	MRKAd5 pol 10 <sup>10</sup> vp	CC1X	4	-	46	256	-
		AW3W	3	-	463	550	-
		AV43	6	-	95	1333	-
4	MRKAd5 pol 10 <sup>8</sup> vp	AW38	1	-	19	30	-
		CC8K	0	-	50	995	-
		CC21	1	-	33	436	-
5	MRKAd5 nef 10 <sup>10</sup> vp	076Q	9	-	-	-	1204
		091Q	4	-	-	-	85
		083Q	0	-	-	-	176
6	MRKAd5 nef 10 <sup>8</sup> vp	00C029	1	-	-	-	114
		98D022	6	-	-	-	170
		98D160	3	-	-	-	198
7	MRKAd5gag+MRKAd5pol+MRKAd5nef 10 <sup>10</sup> vp each	99D251	3	206	15	193	120
		05H	3	135	21	9	638
		00C016	3	26	4	51	23
8	MRKAd5gag+MRKAd5pol+MRKAd5nef 10 <sup>8</sup> vp each	99D215	1	171	18	193	240
		81H	5	73	6	14	243
		12H	8	1140	115	811	719
9	MRKAd5gagpol +MRKAd5 nef 10 <sup>10</sup> vp each	99D211	0	83	56	838	725
		22H	4	385	119	1194	1915
		61H	4	343	11	765	853
10	MRKAd5gagpol +MRKAd5 nef 10 <sup>8</sup> vp each	34H	3	78	19	5	75
		48H	1	65	105	46	43
		70H	5	158	15	220	191

Indicated are numbers of spot-forming cells per million PBMCs against the peptide pools. Mock, no peptides; gag H, fifty 20-aa peptides encompassing p55 sequence; pol-1, 20-aa peptides representing N-terminal half of IA pol; pol-2, 20-aa peptides representing the carboxy-terminal half of IA pol; nef, 20-aa peptides encompassing the entire wild-type nef sequence. Responses to the antigens prior to the first immunization did not exceed 40 sfc/10<sup>6</sup> PBMC.

## WHAT IS CLAIMED IS

- :
1. A recombinant adenoviral vaccine vector at least partially deleted in  
5 E1 and devoid of E1 activity, comprising:
    - a) an adenovirus *cis*-acting packaging region corresponding to from  
about base pair 1 to between from about base pair 400 to about  
base pair 458 of a wildtype adenovirus genome; and
    - b) a gene encoding an HIV protein or immunologically relevant  
10 modification thereof.
  2. A vector in accordance with claim 1 comprising a packaging region  
corresponding to from about base pair 1 to about base pair 450 of a wildtype  
adenovirus genome.
  3. A vector in accordance with claim 1 further comprising nucleotides  
15 corresponding to between from about base pair 3511 to about 3524 to about base pair  
5798 of a wildtype adenovirus genome.
  4. A vector in accordance with claim 3 comprising base pairs  
corresponding to 1-450 and 3511-5798 of a wildtype adenovirus genome.
  5. A vector in accordance with claim 4 which is deleted of base pairs  
20 451-3510.
  6. A vector in accordance with claim 1 which is at least partially  
deleted in E3.
  7. A vector in accordance with claim 6 wherein the E3 deleted region  
is from base pairs 28,133-30,818.

8. A vector in accordance with claim 1 wherein the gene encoding the HIV protein or modification thereof comprises codons optimized for expression in a human.

9. A vector in accordance with claim 1 wherein the vector comprises a  
5 gene expression cassette comprising:

a) a nucleic acid encoding a protein;

b) a heterologous promoter operatively linked to the nucleic acid encoding the protein; and

(c) a transcription termination sequence.

10. A vector in accordance with claim 9 wherein the gene expression cassette is inserted into the E1 region.

11. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette is in an E1 parallel orientation

12. An adenoviral vector in accordance with claim 9 wherein the gene  
15 expression cassette is in an E1 antiparallel orientation.

13. An adenoviral vector in accordance with claim 9 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

14. An adenoviral vector in accordance with claim 13 wherein the promoter is an immediate early human cytomegalovirus promoter.

15. An adenoviral vector in accordance with claim 9 wherein the  
20 promoter is a murine cytomegalovirus promoter.

16. An adenoviral vector in accordance with claim 9 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

17. An adenoviral vector in accordance with claim 9 wherein the transcription termination sequence is a synthetic polyadenylation signal (SPA).

18. A cell comprising the adenoviral vector of claim 1.

19. Recombinant, replication-defective adenovirus particles harvested  
5 and purified subsequent to transfection of the adenoviral vector of claim 1 into a cell line which expresses adenovirus E1 protein at complementing levels.

20. An HIV vaccine composition comprising purified adenovirus particles of claim 19.

21. An HIV vaccine composition of claim 20 which comprises a  
10 physiologically acceptable carrier.

22. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 1 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant,  
15 replication-defective adenovirus.

23. A method according to claim 22 wherein the cell is a PER.C6<sup>®</sup> cell.

24. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of  
20 claim 21.

25. A method according to claim 24 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

26. A method according to claim 25 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

27. A method according to claim 24 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

28. A method according to claim 24 which comprises administering and readministering the adenovirus vaccine vector to the individual.

29. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV gag or an immunologically relevant modification thereof.

30. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV gag protein or immunologically relevant modification thereof.

31. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:

- a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and
- b) a gene expression cassette comprising
- i) SEQ ID NO: 29;
  - ii) a heterologous promoter operatively linked to i); and
  - iii) a transcription termination sequence.

32. An adenoviral vector in accordance with claim 31 wherein the gene expression cassette is in an E1 parallel orientation.

33. An adenoviral vector in accordance with claim 31 wherein the gene expression cassette is in an E1 antiparallel orientation.

5           34. An adenoviral vector in accordance with claim 31 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

35. An adenoviral vector in accordance with claim 31 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

10           36. An adenoviral vector in accordance with claim 31 which is at least partially deleted in E3.

37. A cell comprising the adenoviral vector of claim 30.

38. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 30 into a cell  
15   line which expresses adenovirus E1 protein at complementing levels.

39. An HIV vaccine composition comprising purified adenovirus particles of claim 38.

40. An HIV vaccine composition of claim 39 which comprises a physiologically acceptable carrier.

20           41. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 30 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.



42. A method according to claim 41 wherein the cell is a PER.C6<sup>®</sup> cell.

43. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of  
5 claim 21.

44. A method according to claim 43 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

10 45. A method according to claim 44 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

46. A method according to claim 43 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

15 47. A method according to claim 43 which comprises administering and readministering the adenovirus vaccine vector to the individual.

48. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV pol or an immunologically relevant modification thereof.

20 49. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV pol protein or immunologically relevant modification thereof.

50. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:

- 5 a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and
- b) a gene expression cassette comprising
- i) a nucleotide sequence selected the group consisting of SEQ ID NO: 1, SEQ ID NO: 5 and SEQ ID NO: 7;
  - ii) a heterologous promoter operatively linked to i); and
  - 10 iii) a transcription termination sequence.

51. An adenoviral vector in accordance with claim 50 wherein the gene expression cassette is in an E1 parallel orientation.

52. An adenoviral vector in accordance with claim 50 wherein the gene expression cassette is in an E1 antiparallel orientation.

15 53. An adenoviral vector in accordance with claim 50 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

54. An adenoviral vector in accordance with claim 50 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

20 55. An adenoviral vector in accordance with claim 50 which is at least partially deleted in E3.

56. A cell comprising the adenoviral vector of claim 49.

57. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 49 into a cell line which expresses adenovirus E1 protein at complementing levels.

58. An HIV vaccine composition comprising purified adenovirus  
5 particles of claim 57.

59. An HIV vaccine composition of claim 58 which comprises a physiologically acceptable carrier.

60. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of  
10 claim 49 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.

61. A method according to claim 60 wherein the cell is a PER.C6<sup>®</sup> cell.

15 62. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 59.

63. A method according to claim 62 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with  
20 a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

64. A method according to claim 63 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

65. A method according to claim 62 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

66. A method according to claim 62 which comprises administering and readministering the adenovirus vaccine vector to the individual.

5           67. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV nef or an immunologically relevant modification thereof.

68. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV nef protein or immunologically relevant modification thereof.

10           69. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:

a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs  
15           corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and

b) a gene expression cassette comprising

i) a nucleotide sequence selected the group consisting of  
SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13 and  
20           SEQ ID NO: 15;

ii) a heterologous promoter operatively linked to i); and

iii) a transcription termination sequence.

70. An adenoviral vector in accordance with claim 69 wherein the gene expression cassette is in an E1 parallel orientation.

71. An adenoviral vector in accordance with claim 69 wherein the gene expression cassette is in an E1 antiparallel orientation.

72. An adenoviral vector in accordance with claim 69 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

5           73. An adenoviral vector in accordance with claim 69 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

74. An adenoviral vector in accordance with claim 69 which is at least partially deleted in E3.

10           75. A cell comprising the adenoviral vector of claim 68.

76. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 68 into a cell line which expresses adenovirus E1 protein at complementing levels.

15           77. An HIV vaccine composition comprising purified adenovirus particles of claim 76.

78. An HIV vaccine composition of claim 77 which comprises a physiologically acceptable carrier.

79. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 68 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.

20

80. A method according to claim 79 wherein the cell is a PER.C6<sup>®</sup> cell.

81. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 78.

82. A method according to claim 81 which further comprises  
5 administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

83. A method according to claim 82 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus  
10 vaccine.

84. A method according to claim 81 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

85. A method according to claim 81 which comprises administering and readministering the adenovirus vaccine vector to the individual.

15 86. A multivalent adenovirus vaccine composition comprising recombinant, replication-defective adenovirus particles, wherein the adenovirus particles are harvested and purified from a cell line expressing adenovirus E1 protein, and wherein the particles are harvested subsequent to transfection of the cells with an adenoviral vector or vectors in accordance with claim 9; said vector(s) comprising a  
20 gene expression cassette or cassettes comprising nucleotide sequences encoding HIV proteins selected from the group consisting of:

- a) gag, pol, and nef, expressed independently from three individual vectors;

- b) gag, pol, and nef, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
- c) gag, pol, and nef, expressed via two vectors, one expressing a pol-nef fusion, and another expressing gag;
- d) gag, pol, and nef, expressed via two vectors, one expressing a gag-pol fusion and another expressing nef;
- e) gag, pol and nef, expressed via two vectors, one expressing a nef-gag fusion and another expressing pol;
- f) gag, pol, and nef, expressed via one vector expressing a gag-pol-nef fusion;
- g) gag and pol, expressed independently from two individual vectors;
- h) gag and pol, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
- i) pol and nef, expressed independently from two individual vectors;
- j) pol and nef, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
- k) nef and gag, expressed independently from two individual vectors;
- l) nef and gag, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
- m) gag and pol, expressed via one vector expressing a gag-pol fusion;

n) pol and nef, expressed via one vector expressing a pol-nef fusion;

and

o) nef and gag, expressed via one vector expressing a nef-gag fusion.

87. A multivalent adenovirus vaccine composition in accordance with  
5 claim 86 wherein the gag-pol fusion consists of SEQ ID NO: 39.

88. A multivalent adenovirus vaccine composition in accordance with  
claim 86 wherein the fused sequences have the encoding nucleic acid sequences  
operatively linked to distinct promoters and transcription termination sequences.

89. A multivalent adenovirus vaccine composition in accordance with  
10 claim 86 wherein the fused sequences have the encoding nucleic acid sequences  
operatively linked to a single promoter; and the encoding nucleic acid sequences  
operatively linked by an internal ribosome entry sequence ("IRES").



Original Adenovector Construct:

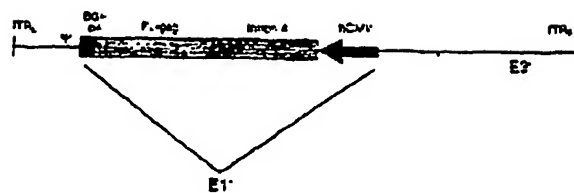


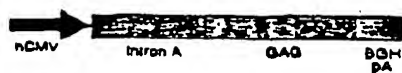
Figure 1: Original HIV-1 gag adenovector.

**Sequence of the open reading frame for FL-qag (human codon optimized)**

atgggtgctagggcctctgctgctgctggttgagctggacaagtgggagaagatcaggctgagggcctgggg  
caagaagaagtaactcagactaaagcacaagctatgctggcctccagggagctggagaggttctgctggaacccctggc  
ctgctggagagctcagtgagggctgagggcagatcctgggccagctccagccctccctgcgaaacaggctctgagg  
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gat (SEQ ID NO: 29)

Figure 2

Old Transgene:



New Transgenes:

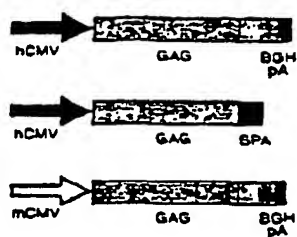
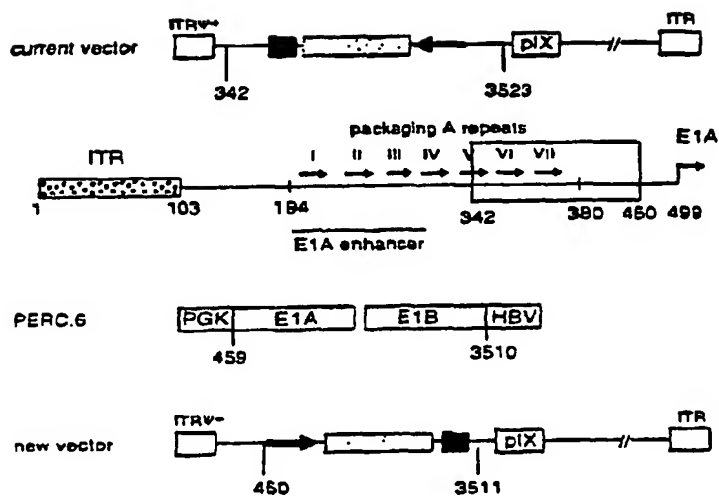
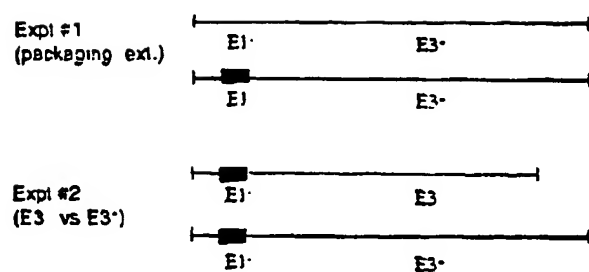


Figure 3: Diagrammatic representation of the original HIV-1 gag transgene and the series of new transgene constructions.



**Figure 4:** Modifications made to the current adenovector backbone in the generation of the new vector.



**Figure 5:** Virus mixing experiments to determine the effects of the addition made to the packaging signal region (Expt #1) and analysis of the effects of the E3 gene on viral growth (Expt. #2). The red bars denote the region of modifications made to the E1 deletion.

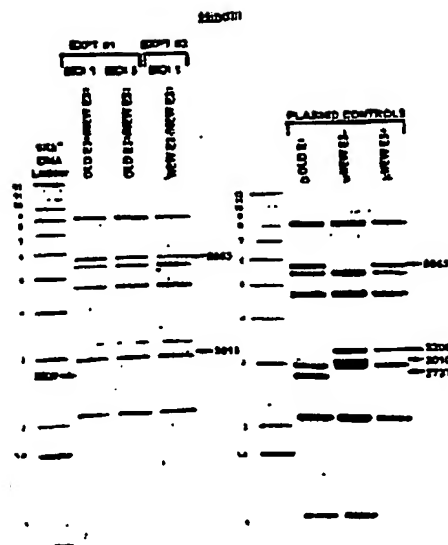
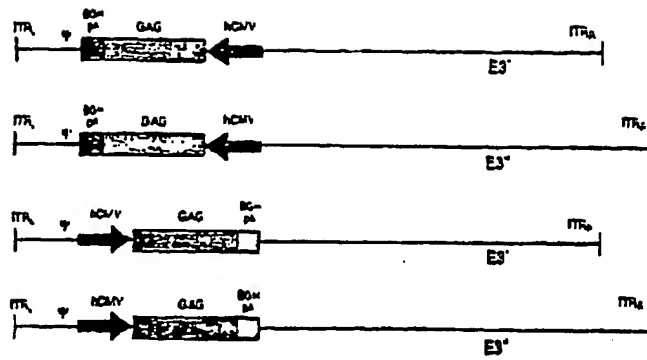
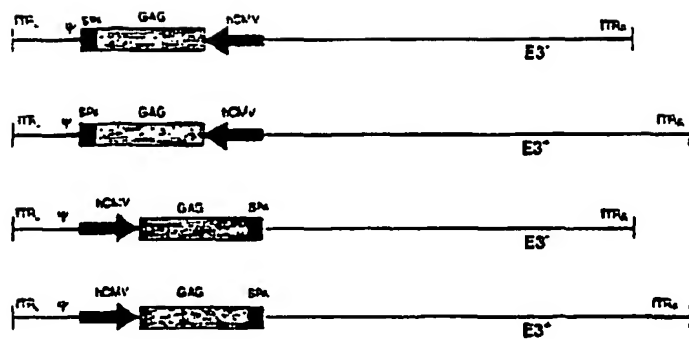


Figure 6: Autoradiograph of viral DNA analysis following viral mixing experiments (expts. #1 and #2) as detailed in the text.

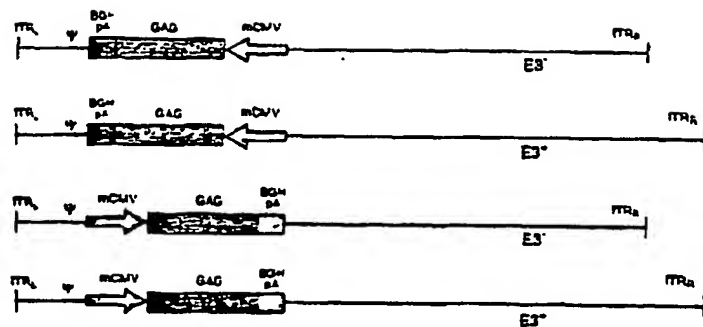


**Figure 7A:** hCMV-FLgag-bGHpA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3<sup>-</sup> and E3<sup>+</sup> backbones were constructed.



**Figure 7B:** hCMV-FLgag-SPA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.





**Figure 7C:** mCMV-FLgag-bGHpA adenovectors constructed within the "MRK" backbones. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.

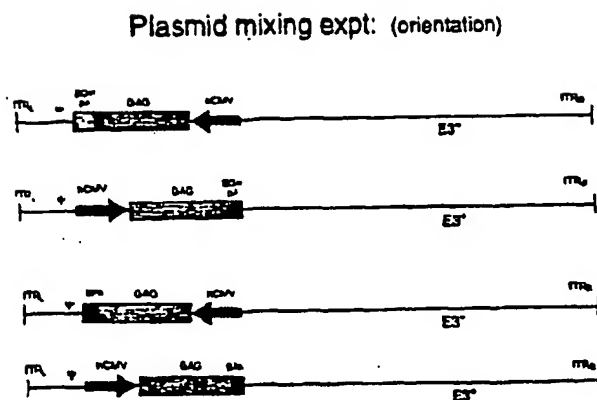
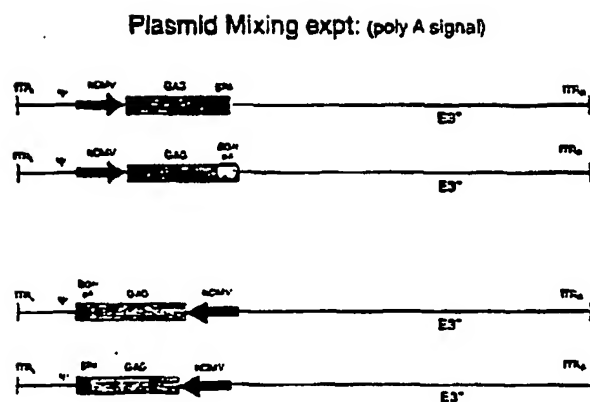


Figure 8A: Effect of transgene orientation



**Figure 8B: Effect of polyadenylation signal**

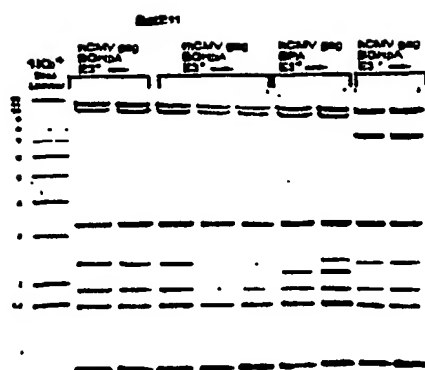
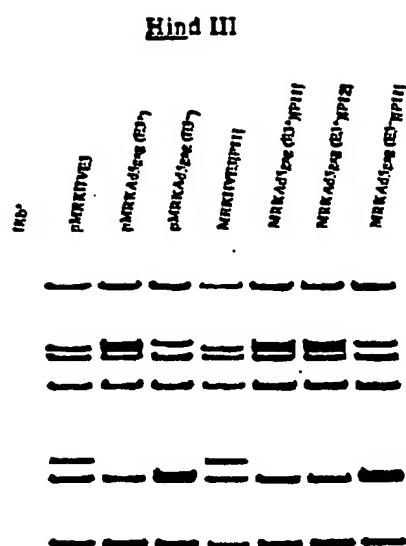


Figure 9: Viral DNA from the four Adgag candidates at P5, following *BstE11* digestion.



**Figure 10: Viral DNA analysis of passage 11 and/or 12 of MRKHVE3, MRKAd5gag and MRKAd5gag(E3-).**

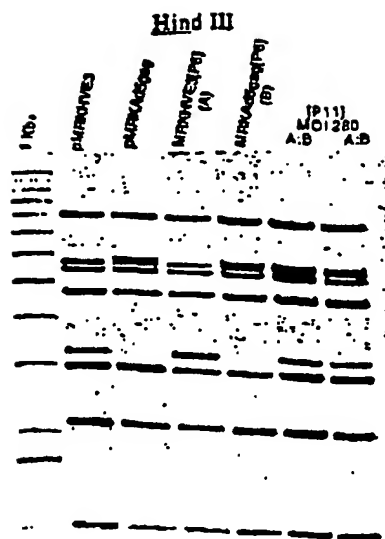
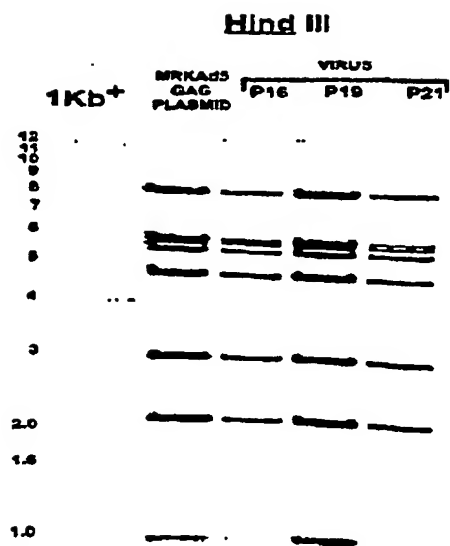
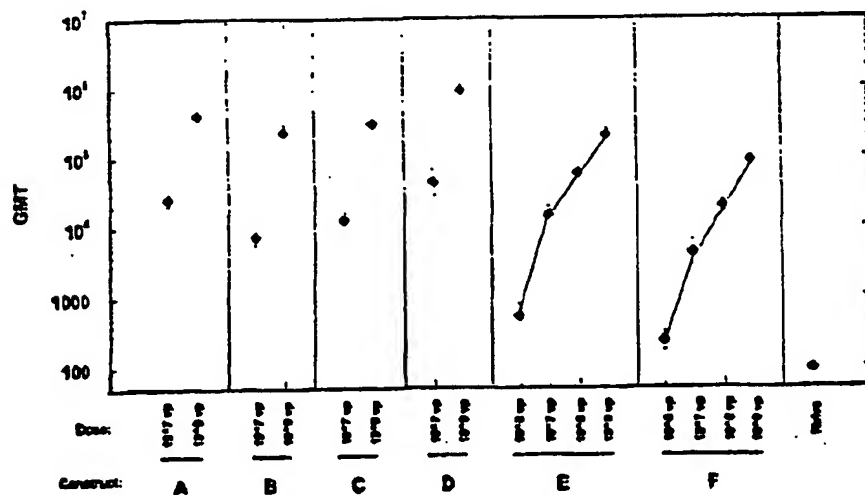


Figure 11: Viral DNA analysis (*Hind*III digestion) of passage 6 MRKHVE3 and MRKAd5gag used to initiate the viral competition study. Last two lanes are passage 11 analysis of duplicate passages of the competition study (each virus at MOI 280 vp).



**Figure 12:** Viral DNA analysis by *Hind*III digestion on high passage numbers for MRKAd5gag in serum containing media with collections made at specified times. The first lane shows the 1 Kb DNA size marker. The other lanes represent pre-plasmid control (digested with Pac1 and *Hind*III), and MRKAd5gag virus continually passaged to P16, P19 and P21 (serum containing media).

13  
**Figure 13.** Serum anti-p24 Levels at 3 Wks post i.m. immunization of balb/c mice (n=10) with Varying Doses of Several Adgag constructs: (A) MRKAd5gag (through passage 5); (B) MRKAd5 E3<sup>+</sup> hCMV-FLgag-bGHPA; (C) MRKAd5 E3<sup>+</sup> hCMV-FLgag-SPA; (D) MRKAd5 E3<sup>+</sup> mCMV-FLgag-bGHPA; (E) research Lot (293 cell-derived) of Ad5HIV-1gag; and (F) clinical lot (Ad5gagFN0001) of Ad5HIV-1gag. Reported are the geometric mean titers (GMT) for each cohort.





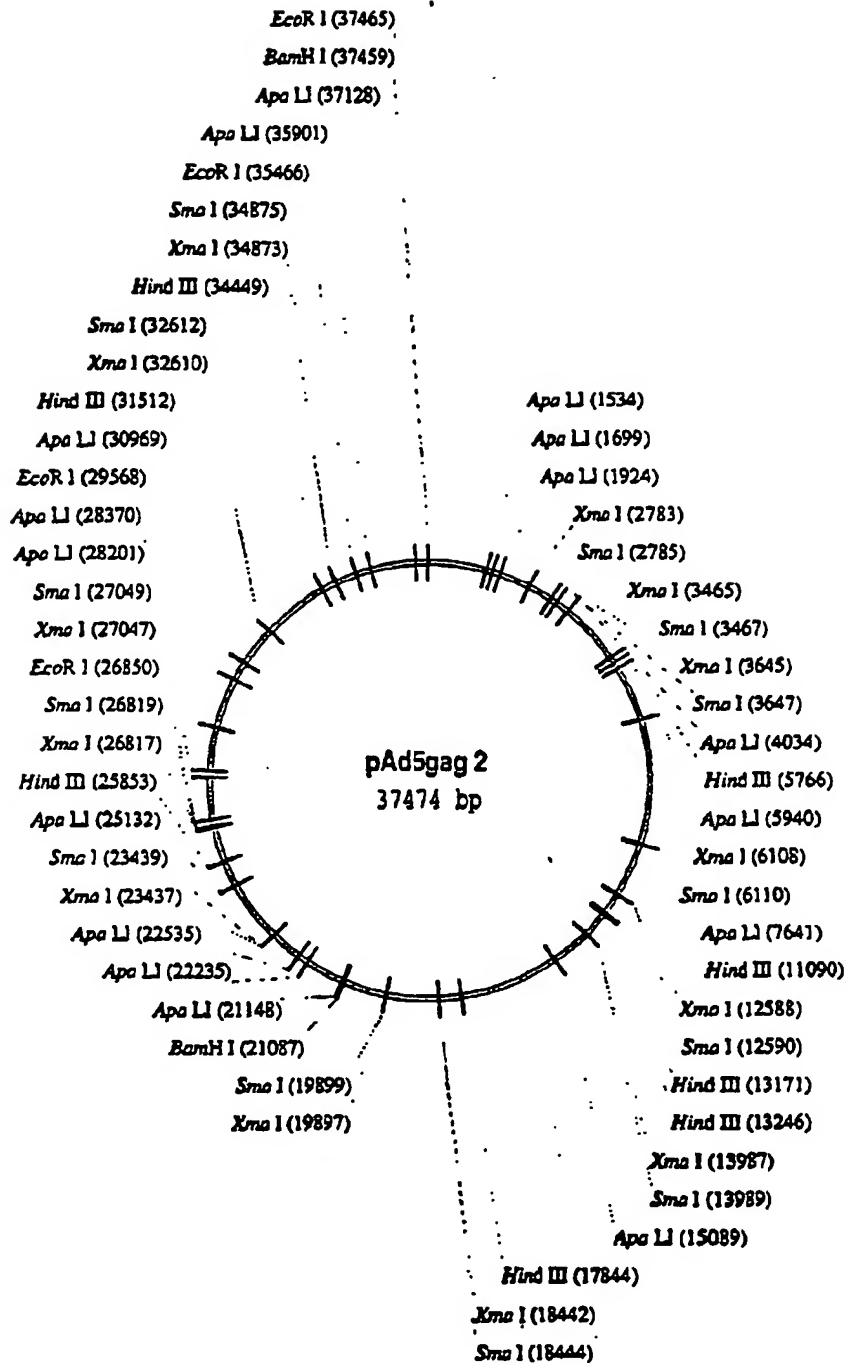


Figure 14

[illegible]

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## PINKA/15q10 MER6B2

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1801	AGGTGTCAC	CCCGCAGGAC	CTGTAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA
1901	TCCAGGCTG	GGGCTGCTG	GACTTTTAT	AGTTTCTTTA	AGTTTCTTTA	AGTTTCTTTA	AGTTTCTTTA	AGTTTCTTTA	AGTTTCTTTA	AGTTTCTTTA	AGTTTCTTTA
2001	AGGTGTCAC	CCCGCAGGAC	CTGTAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA
2101	AGGTGTCAC	CCCGCAGGAC	CTGTAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA
2201	AGGTGTCAC	CCCGCAGGAC	CTGTAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA
2301	AGGTGTCAC	CCCGCAGGAC	CTGTAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA
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2501	AGGTGTCAC	CCCGCAGGAC	CTGTAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA
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2701	AGGTGTCAC	CCCGCAGGAC	CTGTAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA
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2901	AGGTGTCAC	CCCGCAGGAC	CTGTAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA
3001	AGGTGTCAC	CCCGCAGGAC	CTGTAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA
3101	AGGTGTCAC	CCCGCAGGAC	CTGTAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA
3201	AGGTGTCAC	CCCGCAGGAC	CTGTAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA	TTTAAATTA

Figure 15B

[illegible]

20/144

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5101	TCCGCTGCTG GGCCTCTTTC GGCAGATATC TGTCTGCTCA TCTCCAGATG TGTCTGCTCA TCTCCAGATG TGTCTGCTCA
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6201	AGGCTGTAAC TGTCTGCTCA TGTCTGCTCA TGTCTGCTCA TGTCTGCTCA TGTCTGCTCA TGTCTGCTCA TGTCTGCTCA
6301	AGGCTGTAAC TGTCTGCTCA TGTCTGCTCA TGTCTGCTCA TGTCTGCTCA TGTCTGCTCA TGTCTGCTCA TGTCTGCTCA
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Figure 15D

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 6601 TGTCTACTT ATCTGTCCC TTTTCTTTC ACACCTGAT GTACAGATG AATTTTCT GATCTTTTA GTACTTTTG ATCGGAAGC CTTCTCTT  
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Figure 15F

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10001	GTGTGTGCGG GCGCGCGAA GTGTGTGCGG GTGTGTGCGG GTGTGTGCGG GTGTGTGCGG GTGTGTGCGG GTGTGTGCGG GTGTGTGCGG GTGTGTGCGG GTGTGTGCGG GTGTGTGCGG GTGTGTGCGG GTGTGTGCGG
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10101	AAATGTTGAC GTCTGAGACC GTGCAAAAGG AGAGCGCTTA AGCGTGTACT TCGCGCGTTC TCGCGCGTTC TCGCGCGTTC TCGCGCGTTC TCGCGCGTTC TCGCGCGTTC TCGCGCGTTC TCGCGCGTTC TCGCGCGTTC
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10201	GCGTTTCGAG CCGGTATGCG GCGTTTCGCG GCGTTTCGCG GCGTTTCGCG GCGTTTCGCG GCGTTTCGCG GCGTTTCGCG GCGTTTCGCG GCGTTTCGCG GCGTTTCGCG GCGTTTCGCG GCGTTTCGCG GCGTTTCGCG
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10301	TTCGCGTTC TTCGAGCGCG GCGCGCGTTC GCGCGCGTTC GCGCGCGTTC GCGCGCGTTC GCGCGCGTTC GCGCGCGTTC GCGCGCGTTC GCGCGCGTTC GCGCGCGTTC GCGCGCGTTC GCGCGCGTTC GCGCGCGTTC
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	CGAGCGAGCG TCGTGTGCGT TCGTGTGCGT TCGTGTGCGT TCGTGTGCGT TCGTGTGCGT TCGTGTGCGT TCGTGTGCGT TCGTGTGCGT TCGTGTGCGT TCGTGTGCGT TCGTGTGCGT TCGTGTGCGT TCGTGTGCGT
10501	CTGCGCGTTC TCGAGCGCGA GCGTTTCGCG GCGTTTCGCG GCGTTTCGCG GCGTTTCGCG GCGTTTCGCG GCGTTTCGCG GCGTTTCGCG GCGTTTCGCG GCGTTTCGCG GCGTTTCGCG GCGTTTCGCG GCGTTTCGCG
	GAGCGCGAGT ACCTTTCGCG GCGAGCGTTC GCGAGCGTTC GCGAGCGTTC GCGAGCGTTC GCGAGCGTTC GCGAGCGTTC GCGAGCGTTC GCGAGCGTTC GCGAGCGTTC GCGAGCGTTC GCGAGCGTTC GCGAGCGTTC
10601	CCCGCTGCTC AGCAGCGCGA TCGTGTGCGT TCGTGTGCGT TCGTGTGCGT TCGTGTGCGT TCGTGTGCGT TCGTGTGCGT TCGTGTGCGT TCGTGTGCGT TCGTGTGCGT TCGTGTGCGT TCGTGTGCGT TCGTGTGCGT
	GCGCGAGCGG TCGTGTGCGT TCGTGTGCGT TCGTGTGCGT TCGTGTGCGT TCGTGTGCGT TCGTGTGCGT TCGTGTGCGT TCGTGTGCGT TCGTGTGCGT TCGTGTGCGT TCGTGTGCGT TCGTGTGCGT TCGTGTGCGT
10701	CAGCGAGCGA TGTGTATGAC GAGCGCGCGG GAGCGCGCGG GAGCGCGCGG GAGCGCGCGG GAGCGCGCGG GAGCGCGCGG GAGCGCGCGG GAGCGCGCGG GAGCGCGCGG GAGCGCGCGG GAGCGCGCGG GAGCGCGCGG
	GCGGTGTGCT ACCACTAATG CTTGTGCGCG CTTGTGCGCG CTTGTGCGCG CTTGTGCGCG CTTGTGCGCG CTTGTGCGCG CTTGTGCGCG CTTGTGCGCG CTTGTGCGCG CTTGTGCGCG CTTGTGCGCG CTTGTGCGCG
10801	TGAGCGCGAC CCAAGCGTTC AGCTGAGCGG AGCTGAGCGG AGCTGAGCGG AGCTGAGCGG AGCTGAGCGG AGCTGAGCGG AGCTGAGCGG AGCTGAGCGG AGCTGAGCGG AGCTGAGCGG AGCTGAGCGG AGCTGAGCGG
	ACTGCGCGCG GGTGTGCGCG TCGTGTGCGG TCGTGTGCGG TCGTGTGCGG TCGTGTGCGG TCGTGTGCGG TCGTGTGCGG TCGTGTGCGG TCGTGTGCGG TCGTGTGCGG TCGTGTGCGG TCGTGTGCGG TCGTGTGCGG
10901	ATGCGCGATC GAAAGTTTCA GCGTGTGCGG GAGCTGTGCG GAGCTGTGCG GAGCTGTGCG GAGCTGTGCG GAGCTGTGCG GAGCTGTGCG GAGCTGTGCG GAGCTGTGCG GAGCTGTGCG GAGCTGTGCG GAGCTGTGCG
	TACCGCGTTC CTTTTCAGGT GCGTGTGCGG GCGTGTGCGG GCGTGTGCGG GCGTGTGCGG GCGTGTGCGG GCGTGTGCGG GCGTGTGCGG GCGTGTGCGG GCGTGTGCGG GCGTGTGCGG GCGTGTGCGG GCGTGTGCGG
11001	GATTTATGTC CCGCGCGCGA CAGGTGTGCG GAGCGCGCGG GAGCGCGCGG GAGCGCGCGG GAGCGCGCGG GAGCGCGCGG GAGCGCGCGG GAGCGCGCGG GAGCGCGCGG GAGCGCGCGG GAGCGCGCGG GAGCGCGCGG
	CCTTATGAGG GCGCGCGCGT GTGCGCGCGG GTGCGCGCGG GTGCGCGCGG GTGCGCGCGG GTGCGCGCGG GTGCGCGCGG GTGCGCGCGG GTGCGCGCGG GTGCGCGCGG GTGCGCGCGG GTGCGCGCGG GTGCGCGCGG
11101	CGAGGTGCGT ACCGTGTGCG GCGCGCGCGA GCGCGCGCGA GCGCGCGCGA GCGCGCGCGA GCGCGCGCGA GCGCGCGCGA GCGCGCGCGA GCGCGCGCGA GCGCGCGCGA GCGCGCGCGA GCGCGCGCGA GCGCGCGCGA
	CGGTGAGCGA TCGGAGCGCG GCGCGCGTTC GCGCGCGTTC GCGCGCGTTC GCGCGCGTTC GCGCGCGTTC GCGCGCGTTC GCGCGCGTTC GCGCGCGTTC GCGCGCGTTC GCGCGCGTTC GCGCGCGTTC GCGCGCGTTC
11201	GTGAGCGCGG AGCTGTGCTT TATATGTGCG TATATGTGCG TATATGTGCG TATATGTGCG TATATGTGCG TATATGTGCG TATATGTGCG TATATGTGCG TATATGTGCG TATATGTGCG TATATGTGCG TATATGTGCG
	GATTTACCGG TCGGAGCGA ATATGAGTTC GTGTGTGCGG GTGTGTGCGG GTGTGTGCGG GTGTGTGCGG GTGTGTGCGG GTGTGTGCGG GTGTGTGCGG GTGTGTGCGG GTGTGTGCGG GTGTGTGCGG GTGTGTGCGG

Figure 15g



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11301	TCAATTTCAT	AAACATCTCT	CAGATGATAG	TATTTATGTA	GTATTTATTT	AGTTATCT	ACAAATGAT	CGCATCAAC	TATTCCATGC	TATTCCTGTT
11401	AGCTAACTA	TTTGTAGAC	GTCTCTATC	ACTAGTTCT	CTATTTTATC	TTTATGATG	TTTATGATG	GGCTATGTT	ATAGGTATGC	AAATGATCT
11501	CAAGTTTAC	CGCCGCAAG	TATATGATC	CGCTAGCTT	CTATATGTA	ATATGATG	ATATGATG	TTCTATATC	CGATGCTCT	GAATTTCT
11601	GTTCMAAAG	CGGCTTTCT	ATATGATG	GGATATGTA	CTATATGTA	ATATGATG	ATATGATG	TTCTATATC	CGATGCTCT	GAATTTCT
11701	ACCTTAAGC	ACGACCTGC	CGTTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC
11801	CGCTTAAGC	ACGACCTGC	CGTTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC
11901	CGCTTAAGC	ACGACCTGC	CGTTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC
12001	CGCTTAAGC	ACGACCTGC	CGTTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC
12101	CGCTTAAGC	ACGACCTGC	CGTTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC
12201	CGCTTAAGC	ACGACCTGC	CGTTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC
12301	CGCTTAAGC	ACGACCTGC	CGTTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC
12401	CGCTTAAGC	ACGACCTGC	CGTTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC
12501	CGCTTAAGC	ACGACCTGC	CGTTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC
12601	CGCTTAAGC	ACGACCTGC	CGTTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC
12701	CGCTTAAGC	ACGACCTGC	CGTTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC
12801	CGCTTAAGC	ACGACCTGC	CGTTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC	CGCTATGTC

Figure 15H

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12901 GGAATGATG GCTGAAAGCT GCGTTTATG AACCTTTAA TTAATCTAT GATATTTAT GTGATCTTA ACCCTAGTA TTACGCAAT TTGATCTTA GGAATCTTA  
 13001 CCGTACATAC GGAATTTGCG CCGCAATATG TTGATCTAT ATCTATATA CTTATCTAT CCGCTGAT TTAATCTAT TTAATCTAT TTAATCTAT TTAATCTAT  
 13101 ACCGCTATG GCTACGCGCG CCGTATCTAT ACAGCTGATG ATCTATATA CTTATCTAT CCGCTGAT TTAATCTAT TTAATCTAT TTAATCTAT TTAATCTAT  
 13201 TCGGCTATAC CCGTATCTAT CCGCAATATG TTGATCTAT TTAATCTAT GATATTTAT GTGATCTTA ACCCTAGTA TTACGCAAT TTGATCTTA GGAATCTTA  
 13301 TTGCTGCTAA CCGGCTATG TTAATCTAT GATATTTAT GTGATCTTA ACCCTAGTA TTACGCAAT TTGATCTTA GGAATCTTA TTAATCTAT TTAATCTAT  
 13401 AAGGCTGCTT GCGCTGCTG ACAGCTGAT TTAATCTAT GATATTTAT GTGATCTTA ACCCTAGTA TTACGCAAT TTGATCTTA GGAATCTTA TTAATCTAT  
 13501 CTAAGGCTG CCGGCTATG GCGTATCTAT TTAATCTAT GATATTTAT GTGATCTTA ACCCTAGTA TTACGCAAT TTGATCTTA GGAATCTTA TTAATCTAT  
 13601 GATGCTGCTG CCGGCTATG GCGTATCTAT TTAATCTAT GATATTTAT GTGATCTTA ACCCTAGTA TTACGCAAT TTGATCTTA GGAATCTTA TTAATCTAT  
 13701 GATGCTGCTG CCGGCTATG GCGTATCTAT TTAATCTAT GATATTTAT GTGATCTTA ACCCTAGTA TTACGCAAT TTGATCTTA GGAATCTTA TTAATCTAT  
 13801 GATGCTGCTG CCGGCTATG GCGTATCTAT TTAATCTAT GATATTTAT GTGATCTTA ACCCTAGTA TTACGCAAT TTGATCTTA GGAATCTTA TTAATCTAT  
 13901 GATGCTGCTG CCGGCTATG GCGTATCTAT TTAATCTAT GATATTTAT GTGATCTTA ACCCTAGTA TTACGCAAT TTGATCTTA GGAATCTTA TTAATCTAT  
 14001 GATGCTGCTG CCGGCTATG GCGTATCTAT TTAATCTAT GATATTTAT GTGATCTTA ACCCTAGTA TTACGCAAT TTGATCTTA GGAATCTTA TTAATCTAT  
 14101 GATGCTGCTG CCGGCTATG GCGTATCTAT TTAATCTAT GATATTTAT GTGATCTTA ACCCTAGTA TTACGCAAT TTGATCTTA GGAATCTTA TTAATCTAT  
 14201 GATGCTGCTG CCGGCTATG GCGTATCTAT TTAATCTAT GATATTTAT GTGATCTTA ACCCTAGTA TTACGCAAT TTGATCTTA GGAATCTTA TTAATCTAT  
 14301 GATGCTGCTG CCGGCTATG GCGTATCTAT TTAATCTAT GATATTTAT GTGATCTTA ACCCTAGTA TTACGCAAT TTGATCTTA GGAATCTTA TTAATCTAT  
 14401 GATGCTGCTG CCGGCTATG GCGTATCTAT TTAATCTAT GATATTTAT GTGATCTTA ACCCTAGTA TTACGCAAT TTGATCTTA GGAATCTTA TTAATCTAT

Figure 151

[illegible]

Figure 15 J

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16101	CGAGTCATC GCGCGGAGG TCTATGCTC CCGTAAAGG GAGAGTAAZ ATTAAATCT CTGAAGCTA AAGCGGTCA AAGAGAAAA GAAGATATAT GATCCAGTAG CCGCGCTCT AGATACCGG GCGCTCTTC CTG TCTATC TAAATATCTT GATCTTACAT TTGCGGAGT TTTCCTTTT CTTCCTACT
16201	GATGATGAC TTGACAGCA GGTGACACT CTGACCTA CTCTT CTAG GATATATTA CATTGGAAG TTGACGGGT AAGAGTUTT TTGCGACCC CTACTACTTG AACTGCTCT CCGCTTTCAG GACGTATGAT GATGATGCTT GATGCTTTC CAGCTGCGCA TTTCGACAA AAGCGTCTT
16301	CGACCTACCT AGTCTTTTAC GCGCTTTCAG CCGCTTTCAG GATCTTACAG GATGATGCTT GATGCTTTC CAGCTGCGCA TTTCGACAA AAGCGTCTT CGCTGCTGCA TCAGAAATTC GCGCTTTCAG CCGCTTTCAG GATCTTACAG GATGATGCTT GATGCTTTC CAGCTGCGCA TTTCGACAA AAGCGTCTT
16401	CGAGCTCTC GAGGCTTTC CTTAGCGAA GCGCTTTCAG GATCTTACAG GATGATGCTT GATGCTTTC CAGCTGCGCA TTTCGACAA AAGCGTCTT GCTGCGCGAG CCGCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG
16501	CTGACGAGG TGTGCGCGC GCTTTCAGC TGTGCGCGC TGTGCGCGC TGTGCGCGC TGTGCGCGC TGTGCGCGC TGTGCGCGC TGTGCGCGC GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG
16601	AGCGCTACG ACTGAGAGT GCTTTCAGG AATGAGCTT TTTACTGCA CAGTCTTCAG CAGTCTTCAG CAGTCTTCAG CAGTCTTCAG CAGTCTTCAG TGTGCGCGC TGTGCGCGC TGTGCGCGC TGTGCGCGC TGTGCGCGC TGTGCGCGC TGTGCGCGC TGTGCGCGC TGTGCGCGC
16701	GCGCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GCGCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG
16801	GCGCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GCGCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG
16901	GCGCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GCGCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG
17001	GCGCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GCGCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG
17101	GCGCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GCGCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG
17201	ATATGCGCT CAGCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG TATATCGCA GTGACGCGC GAGCGAGG GCGCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG
17301	GCGCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GCGCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG
17401	GCGCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GCGCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG
17501	GCGCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GCGCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG GATCTTTCAG

Figure 15K



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19301	AGACCTAATG	GGCCAGCAAT	CTATCTCAAA	CAGCCCTAAT	TACATCTCT	TTATGAGTAA	TTTATATCTG	CTATATGTAAT	ACATACAGC	GGTAAATATG
19401	CTTTGATATC	GGGCTGTTTA	GATACGCTT	GTCTGTAATA	ATTTATACGA	ATATCTCTTT	AAATATACAA	GATTACATAA	TOTTTCTGTG	CCCATTTATAC
19501	CCATATCTGG	GGGCGCAAC	ATCTAATCTG	ATATATCTGG	TATATTTTGA	AGATATATAC	ACAGATCTTT	CATACCACTT	TTTCTCTCAT	TCCATTTCTG
19601	ATATAGACCA	GGGCGCTTCT	TATCTCAAC	TTATCTCAAC	ATCTAATCT	TTCTCTCTTT	TGCTCTGAAA	ATATGATCTA	AAATGATCTA	AGTTATCTAA
19701	TATCTTCTGC	CATGAAAGA	TACACTTTG	ATCTGTAAT	CTATCTCAAT	CTATCTCAAT	CTATCTCAAT	TTTATATCT	TACTCTCTAC	TTGATCTTAA
19801	TTATCTCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT
19901	TTATCTCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT
20001	TTATCTCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT
20101	TTATCTCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT
20201	TTATCTCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT
20301	TTATCTCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT
20401	TTATCTCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT
20501	TTATCTCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT
20601	TTATCTCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT
20701	TTATCTCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT
20801	TTATCTCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT
20901	TTATCTCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT	GGTATCTCT

Figure 15M

## pMRKAd5q1q1 MR6R2

21001	TTATGTCCAT	GGGGGACTC	ACAGAGCTTG	GGCAAAAGCT	TCCTCACTTC	AACTCCCTCT	ACGGGTGAGA	CATGACTTTT	GAGTGTATTC	CGATGAGTGA	GGGTGAGTGA	GGGTGAGTGA
21101	AAATACAGTA	CCCGGCGTGA	TGCTGTGAC	CGATTTTGA	ATGAGATGTA	TGAGATGTA	TGAGATGTA	TGAGATGTA	TGAGATGTA	TGAGATGTA	TGAGATGTA	TGAGATGTA
21201	GGGACGCTT	CTTTAGCTT	TTTGTGAGT	CTTTGAGT	CTTTGAGT	CTTTGAGT	CTTTGAGT	CTTTGAGT	CTTTGAGT	CTTTGAGT	CTTTGAGT	CTTTGAGT
21301	GGGAGTGA	GAATACAAA	ACAACTTCA	GAATACAAA	GAATACAAA	GAATACAAA	GAATACAAA	GAATACAAA	GAATACAAA	GAATACAAA	GAATACAAA	GAATACAAA
21401	TTGGGCGGTA	AGGGGAGAC	ATTAAGAGC	AGGGGAGAC	AGGGGAGAC	AGGGGAGAC	AGGGGAGAC	AGGGGAGAC	AGGGGAGAC	AGGGGAGAC	AGGGGAGAC	AGGGGAGAC
21501	AGGGGCGCT	TGGGTGTTG	TATTTCTTG	TATTTCTTG	TATTTCTTG	TATTTCTTG	TATTTCTTG	TATTTCTTG	TATTTCTTG	TATTTCTTG	TATTTCTTG	TATTTCTTG
21601	TTGGGTGAG	CGATATTTT	TGGGAGCTA	TGGGAGCTA	TGGGAGCTA	TGGGAGCTA	TGGGAGCTA	TGGGAGCTA	TGGGAGCTA	TGGGAGCTA	TGGGAGCTA	TGGGAGCTA
21701	AGGGGAGCT	GGTATGTTG	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT
21801	AGGGGAGCT	GGTATGTTG	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT
21901	AGGGGAGCT	GGTATGTTG	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT
22001	AGGGGAGCT	GGTATGTTG	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT
22101	AGGGGAGCT	GGTATGTTG	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT
22201	AGGGGAGCT	GGTATGTTG	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT
22301	AGGGGAGCT	GGTATGTTG	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT
22401	AGGGGAGCT	GGTATGTTG	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT
22501	AGGGGAGCT	GGTATGTTG	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT	AGGGGAGCT

Figure 15N



## pNRKAd5gag MER682

22601 ATCTTGCCCT TCTAGACTG CTCTTTACG GCGCTTACG CTTCTTCTT GTTCACATCC ATTCTAATCA CCGTCTCCCT ATTATCATTA ATGCTTCCCT  
 TAGAACCGCA ACCATCTGAC GATTAATCTG CCGCTGATCG GCGCTGATCG GCGCTGATCG GCGCTGATCG GCGCTGATCG GCGCTGATCG GCGCTGATCG  
 22701 GTAGACACTT AGCTGCTCT TCGATTTAG CTGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG  
 CACTCTGCA TTGAGCGCA AGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG  
 22801 CAGCTAGCGC TCAGAGATC GCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG  
 GTCCATCGCG ACCTCTTTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG  
 22901 CATAGCGCG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG  
 GTATCGCGC CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG  
 23001 CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG  
 GTATCGCGC CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG  
 23101 CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG  
 GTATCGCGC CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG  
 23201 CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG  
 GTATCGCGC CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG  
 23301 CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG  
 GTATCGCGC CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG  
 23401 CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG  
 GTATCGCGC CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG  
 23501 CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG  
 GTATCGCGC CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG  
 23601 CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG  
 GTATCGCGC CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG  
 23701 CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG  
 GTATCGCGC CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG  
 23801 CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG  
 GTATCGCGC CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG  
 23901 CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG  
 GTATCGCGC CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG  
 24001 CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG  
 GTATCGCGC CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG  
 24101 CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG  
 GTATCGCGC CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG CCGCTGCTG

Figure 15D



## pHINKAD5gag MERGR2

24201	CGTGGCTGAA GAAAGTGGCA AAGATCTTTG AGATTTTTCG ACCGACACAG AGCTGCTGCTT CAAACCTCTT GAGACGAGAA AACAGCTGAA ATGAGAGTGA GAAAGCGATT GTTTCACGCT TTTTAGAAGC TCCGAGAGAC TGGTCTGCTC TTGATGAGAG GTTTCGCTCTT TTGTGCTCTT TACTTTCAGT
24301	CTCTGGAGTG TGGGTGAAAC TGATAGTTGA GAACTGCTG CAAATGCTAG TAAATATGAG CATGAGATTC AGCCATCTTG GCTAGCCGCG ACTTAACCTTA GAGAGCTTAC AACCACTTTG AGCTCCCACT GTTCTGCTCG GATTTGCTAG GTATCTGAG TGGGTGAAAC GATGAGGCGG TGATTTGGAT
24401	CCCCCAAGG TCAATGAGAC AGCTAGAGAT GATCTGCTCG TATCTGCTCG GATCTGCTCG GATCTGCTCG GATCTGCTCG GATCTGCTCG GATCTGCTCG GAGAGCTTCC AGTACTGCTG TCAATGCTGA CTTGAGTATC AGCTGCTGAG GATCTGCTCG GATCTGCTCG GATCTGCTCG GATCTGCTCG GATCTGCTCG
24501	TACCCGAGAT TGGGAGAGAG CAGCTAGATC AGCTGCTGA AAGCTGCTAG CTGCTGCTAG TGGAGAGAG AGCCAACTTA ATGATGCGCG CAGTCTCTCT ATGCGCTTCA ACCGCTGCTC GTGAGATGCG GAGCCGAGAT TTGCTGCTCG GAGCCGCTGA ACCTCTCTCG TCGCTTTGAT TACTAGCCGCG GTACAGAGCA
24601	TACCGTGGAG CTGAGTGA TCGAGCGATT CTGCTGCTAG CCGAGATTC AGCTGCTAG AGATTAAGCA TTGCACTACA CTTTTCAGCA GCGCTAGCTA ATGCGAGCTC GAACTGAGCT AGCTGCTGA GAAAGCTAG GCGCTCTAGG TCGCTTGA TCTGCTTTCT AAGCTGATCT GGAAGAGCTGT CCGATAGCT
24701	GCGCAGGCTT GCAAGATCTC CAAAGTGGAG CTCTGCAAC TGGTCTCTTA CCTTGAAT TTGCAAGAA ACCGCTCTCG GCAAAAGCTG CTTCATTTCA GCGGTGCGGA CTTTCTAGAG GTTTCAGCTC GAGAGCTGG ACCGAGAGAT GCAAGCTTAA AAGCTGCTTT TGGCGAGACC CTTTTCGAC GAAATAGCT
24801	CGCTCAAGGG CGAGGCGCG CGGAGTACG TCGCGAGCTG TCGCTGCTTA TTCTATGCT ACAGCTGGA GAGCGCATG GCGCTTTGCG AGCAATGCTT GCGAGTCTCC GCTCGCGCGG GCGCTGATCG AGCGCTGAC GCAATGAAAT AAGATAGCA TTGTATGCTCT CCGCGCTGAC CCGCAAGCGG TCGTACAGAA
24901	GAGAGATGCG AACCTGAAAG AGCTGAGAA ACTGCTAAG ACTGCTTGA AGCACTATG GAGCGCTTC AAGAGAGCTT CCGTGGCGCG GCGCTGCTCT GCTCTCTAGG TTGAGATTC TCGAGCTCT TCGAGCTTTT TCGAGCTTTT TCGAGCTTTT TCGAGCTTTT TCGAGCTTTT TCGAGCTTTT TCGAGCTTTT
25001	GACATCAATT TCGCGAGAG CCGCTTAA ACCCTGCAAC AGCTGCTGAG AGCTGCTGAG AGCTGCTGAG AGCTGCTGAG AGCTGCTGAG AGCTGCTGAG CTGAGTAA AGGCGCTTGC GAGCAATTT TCGAGCTTTT TCGAGCTTTT TCGAGCTTTT TCGAGCTTTT TCGAGCTTTT TCGAGCTTTT TCGAGCTTTT
25101	AGGCTGAGG AATCTTCCCG GCGCTGCTG GTGAGCTTTC TACGCTTCTT TCGAGCTTTT ATGCTGCTTA AGTACCGCTA ATGCGCTCGG CCGCTTTGCG GCGCTGCTTA TGGGATGCT TTGAGAACGG CCGTGAAGCA CAGCTGAGG ATGCTGAGAA CAGCGTAAAT TCAATGCGCT TACGCGAGCG GCGCAAGCGC CCGTGAAGCT
25201	CGTCTGCGAG CTAGCGAAT ACCCTGCTTA CCACTGCTAC ATATGAGAG AGCTGAGCGG TTAGGCTTA CTGAGTCTC ACTGCTGCTG CAGCTATTC GAAAGAGCTC GATCGCTTA TCGAGCGAT GGTGAGACTG TATTAAGCTT TCGAGCTCT ACTGCGAGAT GAGCTGAGG TCAACAGGAC GTTGTATATC
25301	ACCGCGGACC GCTCTCTGCT TTGCAATTC CAGCTGCTTA AGCAAGTGA AATATGCTAT ACCCTTGAAG CTGCGGCTCC CTGCGCTGAC GAAGAGCTCT TGGGAGCTCG GAGAGAGCA AAGCTTGAAG GTGAGAGAT TCTTTCAAT TTATAGAGCA TCGAAGCTCG AGCTTCCAG GAGCGAGCTG GTTTTCAGG
25401	GCGCTGCGCG GTTGAAGCTC ACTCGCGCG TCGAGCTCT GGTGAGCTC GTTGAAGCTC GTTGAAGCTC GTTGAAGCTC GTTGAAGCTC GTTGAAGCTC GCGAGAGCGC CAGCTTGAAG TCGCGCGCG AGAGCTGAG CCGAGAGTGA GGTTTGAAG GGTTTGAAG GGTTTGAAG GGTTTGAAG GGTTTGAAG
25501	AGAGCAATTC CCGCGCGCTA ATGCGAGCT TACGCTGCTC GTTATGCTC AGCTGCTGAG AGCTGCTGAG AGCTGCTGAG AGCTGCTGAG AGCTGCTGAG TCTGCTTGA GCGCGCGGAT TACGCTGCTA ATGCGAGCT CAGTATGCT ATGCGAGCT AGAGAGCTT AGAGAGCTT AGAGAGCTT AGAGAGCTT AGAGAGCTT
25601	TTTCTGCTAC GAAGAGAG GCGCTTTTAC TTGAGCTCC TTGAGCTCC TCAAGCTGAG CAGCTGAG CAGCTGAG CAGCTGAG CAGCTGAG CAGCTGAG AAGAGAGT CTTTCTGCT CCGCGAGAG AGCTGAGAG TCAAGCTGAG TCAAGCTGAG TCAAGCTGAG TCAAGCTGAG TCAAGCTGAG TCAAGCTGAG

Figure 15P

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25701 GGGGCGCTTGC TTCCGAGGAT GGCAGCTAAA AATGAGCTAC ATCTGAGAG ACTGAGAGAG GTTAGAGAG GAACTTTCCG GAACTTTCCG AATGAGCTAC AATGAGCTAC AATGAGCTAC AATGAGCTAC AATGAGCTAC  
 CCGCGGAGCG AGGGTCTCTA CCGGCTTTT TTCTTCAGG TTCTTCAGG TTCTTCAGG TTCTTCAGG TTCTTCAGG TTCTTCAGG TTCTTCAGG TTCTTCAGG TTCTTCAGG TTCTTCAGG TTCTTCAGG TTCTTCAGG TTCTTCAGG  
 25801 TCGACGAGCA GAGAGAGGAC ATGATGAGAG ACTGAGAGAG GTTAGAGAG GAACTTTCCG GAACTTTCCG AATGAGCTAC AATGAGCTAC AATGAGCTAC AATGAGCTAC AATGAGCTAC AATGAGCTAC AATGAGCTAC  
 AACTGCTCTT CTTCTCTCTG TACTGCTCTT TACTGCTCTT TACTGCTCTT TACTGCTCTT TACTGCTCTT TACTGCTCTT TACTGCTCTT TACTGCTCTT TACTGCTCTT TACTGCTCTT TACTGCTCTT TACTGCTCTT TACTGCTCTT  
 25901 CGCATTTCCC TCGCGCGCGC CCGAGAAATC GGCAGAGAGT GGCAGAGAGT GGCAGAGAGT GGCAGAGAGT GGCAGAGAGT GGCAGAGAGT GGCAGAGAGT GGCAGAGAGT GGCAGAGAGT GGCAGAGAGT GGCAGAGAGT GGCAGAGAGT  
 GCGTAAGCGG AGCGCGCGCG GCGTCTTTAG GCGTCTTTAG GCGTCTTTAG GCGTCTTTAG GCGTCTTTAG GCGTCTTTAG GCGTCTTTAG GCGTCTTTAG GCGTCTTTAG GCGTCTTTAG GCGTCTTTAG GCGTCTTTAG GCGTCTTTAG  
 26001 AACGTAAGAT GGCAGAGAGT TCGAGAGAGT TCGAGAGAGT TCGAGAGAGT TCGAGAGAGT TCGAGAGAGT TCGAGAGAGT TCGAGAGAGT TCGAGAGAGT TCGAGAGAGT TCGAGAGAGT TCGAGAGAGT TCGAGAGAGT TCGAGAGAGT  
 TCGAGAGAGT TCGAGAGAGT TCGAGAGAGT TCGAGAGAGT TCGAGAGAGT TCGAGAGAGT TCGAGAGAGT TCGAGAGAGT TCGAGAGAGT TCGAGAGAGT TCGAGAGAGT TCGAGAGAGT TCGAGAGAGT TCGAGAGAGT  
 26101 GCGGAGAGCA GCGGAGAGCA GCGGAGAGCA GCGGAGAGCA GCGGAGAGCA GCGGAGAGCA GCGGAGAGCA GCGGAGAGCA GCGGAGAGCA GCGGAGAGCA GCGGAGAGCA GCGGAGAGCA GCGGAGAGCA GCGGAGAGCA GCGGAGAGCA  
 GCGGAGAGCA GCGGAGAGCA GCGGAGAGCA GCGGAGAGCA GCGGAGAGCA GCGGAGAGCA GCGGAGAGCA GCGGAGAGCA GCGGAGAGCA GCGGAGAGCA GCGGAGAGCA GCGGAGAGCA GCGGAGAGCA GCGGAGAGCA  
 26201 CCGTAAGATC CCGTAAGATC CCGTAAGATC CCGTAAGATC CCGTAAGATC CCGTAAGATC CCGTAAGATC CCGTAAGATC CCGTAAGATC CCGTAAGATC CCGTAAGATC CCGTAAGATC CCGTAAGATC CCGTAAGATC CCGTAAGATC  
 GCGATTTGAG GCGATTTGAG GCGATTTGAG GCGATTTGAG GCGATTTGAG GCGATTTGAG GCGATTTGAG GCGATTTGAG GCGATTTGAG GCGATTTGAG GCGATTTGAG GCGATTTGAG GCGATTTGAG GCGATTTGAG GCGATTTGAG  
 26301 TAGCAGAGCT CTGAGAGAGT CAGAGAGAGT CAGAGAGAGT CAGAGAGAGT CAGAGAGAGT CAGAGAGAGT CAGAGAGAGT CAGAGAGAGT CAGAGAGAGT CAGAGAGAGT CAGAGAGAGT CAGAGAGAGT CAGAGAGAGT CAGAGAGAGT  
 AGCTTTCTGA GAGTCTTTAG GAGTCTTTAG GAGTCTTTAG GAGTCTTTAG GAGTCTTTAG GAGTCTTTAG GAGTCTTTAG GAGTCTTTAG GAGTCTTTAG GAGTCTTTAG GAGTCTTTAG GAGTCTTTAG GAGTCTTTAG GAGTCTTTAG  
 26401 GTTAGAAGCA GATTTTCTAT CACTTTCTAT CACTTTCTAT CACTTTCTAT CACTTTCTAT CACTTTCTAT CACTTTCTAT CACTTTCTAT CACTTTCTAT CACTTTCTAT CACTTTCTAT CACTTTCTAT CACTTTCTAT CACTTTCTAT  
 GATTTTCTAT GATTTTCTAT GATTTTCTAT GATTTTCTAT GATTTTCTAT GATTTTCTAT GATTTTCTAT GATTTTCTAT GATTTTCTAT GATTTTCTAT GATTTTCTAT GATTTTCTAT GATTTTCTAT GATTTTCTAT GATTTTCTAT  
 26501 CCGGAGAGT CCGGAGAGT CCGGAGAGT CCGGAGAGT CCGGAGAGT CCGGAGAGT CCGGAGAGT CCGGAGAGT CCGGAGAGT CCGGAGAGT CCGGAGAGT CCGGAGAGT CCGGAGAGT CCGGAGAGT CCGGAGAGT  
 GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT  
 26601 CTAGTTTCTC GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT  
 GATCAGAGCG GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT  
 26701 GAAATTTCCA GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT  
 CTTTAAGGAT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT GCGGAGAGT  
 26801 GACCCAGCAT GATATCTCGG GTCAAGCGAA TACGCGCGCA CCGGAGAGT TACGCGCGCA TACGCGCGCA TACGCGCGCA TACGCGCGCA TACGCGCGCA TACGCGCGCA TACGCGCGCA TACGCGCGCA TACGCGCGCA TACGCGCGCA  
 CTGCGGCTGA CTATAGGCGC CAGTTGCTTT ATGCGCGCGT GCGGAGAGT TACGCGCGCA TACGCGCGCA TACGCGCGCA TACGCGCGCA TACGCGCGCA TACGCGCGCA TACGCGCGCA TACGCGCGCA TACGCGCGCA  
 26901 TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA  
 AGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA  
 27001 TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA  
 AGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA  
 27101 AGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA  
 TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA  
 27201 TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA  
 AGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA TCGCGGCTGA

Figure 156

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27301 CCTCCCGGCC ACTATCCGGA TCATTTTAT CTATACCTT ACCTTTTATA GCACTGCGGT GAGCGCTACG ACTGAAATGTT AGTGTGAGAG CGAGAGCAAC  
 GAGAGCGCGG TGTAGAGCT AGTTAATATA GATTGAAAC TGTATATTT CCTGAGCTTC CTGCTCATTC TACTTTTACA TTCACTCTTC COTCTCTTT  
 27401 TGGGCTTGAA AGACTGTGTC CACTGTGTC CCTACATCT CTATCTGTC GACTTCTGTA AGTTTGTCTA CTTTGAAATG CCGCGAGTAT ATATCTGAGT  
 AGCGGAGCTT TGTGAGACAG GTGAGAGCG CCGTGTCTAC GAAATATGCG GTGAGCGCAC TCANAAAGAT GAACCTTAC GAGTCTCTAG TATGCTCTT  
 27501 CCGCGCGCAC GCGGTCCGCG TTACCGCGCA GCGGAGCTT GCGCTTACG TCATTCGGA TCATTCGCTT CAATCGCTC GCGCGCGAG ATCAACTGCG CCGTCCGCT  
 GCGCGCGCGT CCGAGCGCG ANTGCGCGT CCTCTCTGAA CCGCGATCG ACTAACTGCT <sup>Ngli</sup> GCGCGCGCT GCGCGCTC CAATCGCTC GCGCGCGAG ATCAACTGCG CCGTCCGCT  
 27601 CCTCTGTGTC TCATCTGAT TTGCACTGT CCTAACCGT GATTACATCA AGATCTTGT TGCATCTCT TGCATCTCT TATATATAT AGAATTTAA  
 GCGAGACAG AGTGACATA AGCTTGACA AGCTTGACA CTATTTTGT TCTAGANCA AGCTTACAG CAGACTCAT ATTATTTATG TCTTTTAT  
 27701 AATATCTGCG GCTCTTATCG CCATCTGTA AGCTCCAGC TTCTACCGC CTTCAGCAG CCGAGCGAA CCTTACCTCG TACTTTTAC ATCTCTCG ATCTCTCG  
 TATATGAGCC CAGGATAGC GTTAGACAT TTGCGTGGC AGAATGTGCG GGTTCGCTT GGTTCGCTT GGTTCGCTT GGTTCGCTT GGTTCGCTT GGTTCGCTT  
 27801 CTGCAATTTA CAACAGTTC AGCCAGAGC GATGAGTGT ACTATAGAAC CTCTCCGCG TCACTACTC CATAGTACTC ATAGCAAGC TCCCTTACTC  
 GAGACTTAAT GTTGTCAAG TTGGGTCTCG CTCCTACTGA TCTCTCTTG GAGAGCTCG AGTGTATGAG TCACTACTC TCCCTTACTC TCCCTTACTC  
 27901 CCGGAGAGCT AGAGTGGT GAGCGCGCG CTACCCAGC CTACCGCTG CTACCGCTG CTACCGCTG CTACCGCTG CTACCGCTG CTACCGCTG CTACCGCTG  
 GCGGCTTCCA TCTCTAGCA GTGCGCGCG AGCTGTGCG GATGCGGAG TCGCATTTG TCTGAAAGG GCTGTCTCG AGTATTTGAG ACAAATGCTC  
 28001 AACAGAGGT GAGCTTGA AAACCTTAG GTATTTAGCG GAATTTAGCG GTATTTAGCG GTATTTAGCG GTATTTAGCG GTATTTAGCG GTATTTAGCG  
 TTGCTCTCCA CTGAACTT TTGGATATC CATTAATCG TTTCGCGT GATGAGAGC CAATATCTG TTATTTCTG TTAGTTCTG TTAGTTCTG  
 28101 TCACTGTCT CTAGATCG GTTGTGCT ATCTCTGCT TTGTGATCT CTATTTCTT ATACTAGC TTCTCTGCT AGGCTGCG GCGTCTCT  
 AGTGCAGAGA GATCTTAGC CCAACCCCA TAGAGAGCG AACCTTAGC GAATATGAG TATGATTTG TATGATTTG TATGATTTG TATGATTTG TATGATTTG  
 28201 TCGACATTTG CATTTATTT CAGCTTTTA AGCGTGGG TCGCACCA AGATGATTA GTACATATC GTAGCTTAC TCAGCTTAC TCAGCTTAC TCAGCTTAC  
 AGCTGTAAAC GTAAATACA GTGAAATAT TTGCGAGCG AGCGTGGT TCTACTATC CATGATTTG GATGAGAGC AGTGCAGAGC CAGTCTGCT  
 28301 GATACAGCC AAAGGTGTA TTATAGGAG CAGGCTGTA AGTTTACTT CCGAGCTGA GCTATGAT GCACTACT CTATATGAT TATATATG ACCACAG  
 GATGCTGCG TTCTCCAGT AAATTTCTC GTTGGAGAT TACATGTA GCTTGGACT CCAATTTCTA GGTGTTGAG ATATTTTACG TGGTCTCT  
 28401 ATGAAAGCT CATTATTC CACAAACA AAATTTGCA GTATCTGTT TATGCTATTT TCAAGCGAG TCAGCTTAC GATATATG TTAGCTTT  
 TACTTTTGA CAAATAGCG GTGTTTTT TTATACCTT CATAGCAAA ATAGCAAAA CCGTCTGCT ACTGTGAT CTCAATATC AATGCTCAA  
 28501 CCAAGGTAAA AGTCATAAA CTTTTATGTA TACTTTTCA TTATTTGAA TGTGAGAT TCCATGTC ATGAGCAAC AGTATATGTT GTGCGCGCA  
 GGTGCGATTT TCAATATTT GAATATCAT ATGAAAGAT AAATATCTT ACAGCTGTA ATGATATG TACTGTTG TACTGTTG TACTGTTG TACTGTTG  
 28601 CAATATTTG TCGAAGAC TGGCACTTC TGTGCTACT CTATGCTAT TACAGTCTC TCTTGTCT GTACCTCTCT CTATTTTAA TACANAGCA  
 GTTTTATGAC ACCTTTTGT ACCGTAAAG AGAGTGCAC CATAGATTA ATGTCAGAG CCAACCGAG CATGCGATCA GATATATTT ATGTTTCT  
 28701 GAGGAGCTT TATTGAGAA AGCAATGTC CTATATTTAC TATGTTACA TATGTTACA TATGTTACA TATGTTACA TATGTTACA TATGTTACA  
 CTGCGTGA ATAACTCT TCTTTTACG GAATTAATG ATTCATATTT TCGATTTAG TGTGATTTA CCAATATGCT GGTATGCTG TCTTTTAT  
 28801 AAAGTTAGC ATTAATTTA GATAGATTT TAAACCGCG GTCATTTTC TCTCATATC TCTCATATC TCTCATATC TCTCATATC TCTCATATC  
 TTTTCAATG TAATATTAAT CTATCTTAA ATTTGCGCG CCAATAGAG ACAGTTATG GTAAGCGAG TTGTTTACTG AGATACAGC TATACAGCT

Figure 1SR

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28901 GCGCTACAC CTTGAGTCA GCTTCTCTG ATGTAGCAT CTACTTTTG CAGGACCTG TCCGCGCGAT TGTCTCCAGT CCACTACAT CCACTCCACTC  
 CCGCATCTG GAACTCTAGT CCGAGCGATC TACAGTGTGA CACTGAAAC GGTCTGTGAC AGCGCGCTA ACAGCGTCA GGTGTAGTCT CATTGTGTTT  
 29001 TACAGCGAT GACGACACA ACCAGCGTG CCGCGCTAC CTGACTTACA TTACACAMA ATACACCCA AGTTCTGCC TTGTTCANAT ACTGTATATA  
 ATTCTCTCA CTGCTGTCT TGTGTGCGC GCGCGCATG GCTGTAACT GCTGTAACT TATGTGTGT TCANAGCGG AACACTTAT TCACTCCATCT  
 29101 CTGCGCATG TGTGTGCTT CCACTGCTT TATGTGTGA TGTCTTATTA TTATGTGTCT CATCTCTGCT CTANAGCGCA AACGCGCGG AGCTCCCATC  
 GAGCGCTAC ACCGCAAGA GCTATGCGGA ATACAAATAT AGGTAAATAT ATACACATA GTACAGCAGT GATTTGCGCT TTGCGCGCGC TCGTGTGTAG  
 29201 TATGTGCTA TCACTTCTCT ACACCGAAC AAATGATGAA TGTATGATAT GTACGCTATG AACACATAT TCTTTCTCT TACGTGTATGA TTAAATGAGA  
 ATATCGCGT AGTAACAGTA TGTGTGTTTG TTACTACTT AGGTATCTTA CCGCGCTGAC TTGTGTGACA TGTGTGATAT ATGTCTACT ATTTTACTCT  
 29301 CATGATCTCT CCACTTTTGA TATTAAGTAC CTTGTGCGC CTTTGTGAG CTTGCTGAC ATTTGCGCG GTTCTGACA TCGAACTAGA CTGATCTA  
 GTACTAGAGA GCTCAAAAT ATATGACTG GAGACAGCG GAGCGATG TACCGAGCG CAAAGATGT AGCTTCATCT GACTTATCT GACTTATG.T  
 29401 CCGTTCACAG TCTATTTCT TACGCGAT GTACCGCTCA GCTCATCTG CAGCGCTGAC ACTGTGCTA TCGCTTTAT TCGCTTTAT CCACTGCTAT GACTGCTAT  
 CCGAGCTCT AGTAACAGA AAATGCTTAA CAGTGGAGT GCTGATGAG GCTGTGAGT TGTACAGT TGTACAGT AGCGAAATA GCTTACGTRA CCGACCGTA  
 29501 GTGTGCTT TGTATCTC AGACACTAC CCACTGACAG GTACAGACT ATAGCTGAG TGTGTAGAT TCTTTATTA TGTAAATTA TGTAAATTA TGTGCTTT  
 CACAGCGAA AGTATAGAG TGTGTGCTG CCGTCTGCT CCGTCTGCTA TATGAGCTG AGATCTTA AGAATTAAT ACTTTAAAT ACTTAAAT  
 29601 CTGCTGATTA TTGCACTCT ATCTGCTTT TGTGTGCGA CCGTCAAGC TCAAGAGAT ATATGAGA GATCTCTG TATGTAGAT ATTCTGAT  
 GAGCTTAT AACGTGGA TAGAGGAA ACAGCGCT GAGCTGCG AGTTCTGTA TATGTAGCT CTAAAGTAC ATATGCTTA TAAAGCTTA  
 29701 GCTACAGTA AAAGCGAT CTTTCCGAG CCGGTATTA TCGATCAT TCGTGTGAG TGTCTGCG TACCATCTA GCGTACCTA TATATCTA  
 GATTTTACT TTTTGTGCTA GAAAGCTC GAGCAATAT AGTGTAGT AGACATAC AGACAGCTC ATGTAGAT CCGATCTAT ATATGCTAT  
 29801 CCGTGTGAT GCGTGTGAG CAGTGTGAG CCACTTTCC CCGCGCTGCT TATGTCTCA CCGTGTGCG TGTGTGCGG GCGCTTTCTC  
 GAGCTGTA CCGACTTCT GTATCTAG GCTGTGAG GCTGTGAG GCTGTGAG ATACAGAGT GAGCTGTG TACAGCTG AACAGCGCG GCGTAAAT  
 29901 CCGCGATC AGCTGTGCG ACTTGTGCG AGCTGTGCG AAATGCTA CTTTATCTA ACAGAGAG. ATGCTGCA CCGTATCT AGAATGAC  
 GGTGTGAG TCGAGCGCG TCGAGCGCG TGTGTGAG TTTATGAT GAAATGAT TGTGTGCT TACTGCTCT TACTGCTCT GAGTCTAGA TCTTACTCT  
 30001 GAGATTTTA CAGAGCGCG CCGTGTGAG AGCGCGCG CAGCGCGCG AGACAGCG AGACAGCG AGCTGTGAG CATGTGAT TGTGTGAG TGTGTGAG  
 CCGTGTGAG GTGTGTGCG GAGCGCTCT TGTGTGCG GGTGTGCG GGTGTGCG TACTGTCT TACTGTCT TACTGTCT TACTGTCT TACTGTCT  
 30101 TATGTGTGT CCGTGTGAG AGCTGTGAG AGCTGTGAG AGCTGTGAG AGCTGTGAG AGCTGTGAG AGCTGTGAG AGCTGTGAG AGCTGTGAG  
 CCGTGTGCG ATAGAGAG GAGCTGTCT TCGTGTCTA CCGTGTCTA CCGTGTCTA CCGTGTCTA CCGTGTCTA CCGTGTCTA CCGTGTCTA  
 30201 GAGTGTGAG CAGTGTGAG CAGTGTGAG CAGTGTGAG CAGTGTGAG CAGTGTGAG CAGTGTGAG CAGTGTGAG CAGTGTGAG  
 CTTTACAG CAGTGTGAG CAGTGTGAG CAGTGTGAG CAGTGTGAG CAGTGTGAG CAGTGTGAG CAGTGTGAG CAGTGTGAG  
 30301 CCGTGTGAG TTTTGTGAG CCGTGTGAG CCGTGTGAG TTTTGTGAG TTTTGTGAG TTTTGTGAG TTTTGTGAG TTTTGTGAG  
 GAGTGTGAG TTTTGTGAG TTTTGTGAG TTTTGTGAG TTTTGTGAG TTTTGTGAG TTTTGTGAG TTTTGTGAG TTTTGTGAG

Figure 155

## pMRKad5.gag HERG82

30401	AAATTTCTGT CCAGTTTATT CAGCAGCACC TCCCTGTGCT CCTTTCAGCT CTGTATTATG AGCTTCTGCT TGGCTGCAAA CTTCCTCCAC AATCTAAATG	
30501	TTTAAAGACA GGTCAATATA GTCTGTGTGG AGTAAAGTGA GATATATAGG TTGTAAAGAG ACCGACGTTT GAAAGAGTGG TTAGATTATAC	
30601	GAATGTCAGT TTCTCTCTGT TCTGTGCTAT CCGTACCCAC TATCTTCATG TTCTTTATGA TGAAGGTGCT AGACCTGCT GAGATACCT TCACCTCCCT	
	CTTACAGTCA AAGAGACACA AGATAGGTA GATATATGTA ATAGATATG ACACACTGCT ACTTCTGCTG TCTGTGCTGA CTTCCTATGGA AATTGCTG A	
	GTATCCATAT GACACGAAA CCGTCTCTCC ACTGTGCTG TTCTTATGCT CCGCTTCTGT ATCTCCCAAT GGTTTTCAAG AGATGCTGCC TGGGTACT :	
	CATAGGTATA CTGTGCTTT GGCAGGAGG TTGACACGGA AAGATATGAG GAGGAAACA TAGGGGTTTA CCAAAAGTTC TCTGAGGAGG AGCCCATGAG	
	SgII	
30701	TCTTTGCCCC TATCCGACC TCTAGTTACC TCCATAGGCA TGTGTGCTCT CAATATGTC AACTGTCTCT CTCTGACGGA GCGCGGTCAC CTTTACCTCT	
	AGAAAGCGGG ATAGGCTTGG AGATCAATGG AGCTTACCTG ATGTACGCTA GTTTTACCGG TTGCGGAGGA GAGACCTGCT CCGGCGCTTG GAATGAGG : 1	
30801	AAATGTATAC CACTGTGAGC CCACCTCTCA AAAAAGACCA GTTAAATATA AACTGTGAAA TATCTTATCC CCTCACAGTT ACCCTAGAGG CCTTACTCT	
	TTTTACATTG GTGACACTGG GTTGTGAGCT TTTTGTGCTT CAGTTGTAT TTGACCTTTT ATAGAGCTGG GAGGTGTCGA TGGAGTCTTC GGTATTGACA	
30901	GCTGTGCCCC GCACCTCTAA TGTGTGCGGG CAACACACTC ACCTTCAT CACAGGCCCC GTTAAAGCTGG CAGGACTTCA AACTTATGAT TCCACCCCA	
	CCGACGCGGG CGTGAGATT ACCAGCGCCC GTTGTGTGAG TTGTAGCTTA GTGTGCGGG CCAATGCGAC GTGCTGAGT TTGAATGTA AGGTGCGT : 1	
31001	GCACCTCTCA CAGTGTGAGA AGGAAAGCTA GCGCTGCAAA CATCAAGCCC CCTCACACC AGCGATAGCA GTACCTTTAC TATCACTGCC TCAACCCCTT	
	CCGTGCGAGT GTACAGTCT TCTTTTGAT CCGGAGCTTT GTATGTGCGG GAGGTGCTGG CATGTGATGT ATGTGTGCGG AATGTGCGGA	
31101	TAACTACTGC CACTGTGAGC TTGGGCTATG ACTGTGAGA GCGCATTTAT ACACAAATGG GAACACTAGG CACTTATGCT GCGGCTCTCT TGTCTGTN	
	ATTGATGAGC GTGACCATGG AACCGGTAC TGAATCTTCT CCGTAAATTA TGTGTTTATG CTTTGTATGC TGAATTTATG CCGGCAAGGA AGGTATCTT	
31201	AGAGGACCTA AACTTTTGA CCGTAGCAGC TGTTCAGGT GTGATTTTGA ATATATCTTC CTGTCAACT CTTCGACTGG GAGCTTTGG TTTTGATTA	
	TCTGTGAT TTGTGAACT GCAATCTTTG ACCAGGTCCA CACTGTAAAT TATTATGAG GAGGTTTGA TTTCATGAG CTGTGAAACC NAACATA	
31301	CAGGCAATA TGCACCTTAA TGTACAGGA GACTTAAGCA TTGATTTCTA AACAGAGCC CTTATACTTG ATGTATGTA TCGGTTTGTAT GCTCAAACT	
	GTTCCTTAT ACTTGAAT ACTGCTGCT CCGTATCTCT AACTAAGAT TTGTGCTGG GAATATGAC TADATCAAT AGGCAAACTA CAGTTTTH	
31401	AACTAAATCT AAGCTATGGA CAGGCTCTTC TTTTATATA CTACGCCCAC AACTGTGATA TTACTAGAA CAAGGCGCTT TACTTGTGTA GAGCTTCA	
	TTGATTTAGA TTCTGATCT GTGCGCGGAG AAATATATT GAGTGGGTG TTGAACCTAT AATGATGTT GTTTGCGGAA ATGTACAAAT GTGCAAGTT	
	HindIII	
31501	CAATTCGAAA AAGCTTGAGG TTAACTTAG CACTGCCAAG GATTTGATGT TTGACCTTAC AGCGATAGCC ATTAATGCGG GAGATGCGCT TGAATTTG	
	GTTAAGGTTT TTGTGACTGC AATTGATATC GTGACGTTTC CCACTATCA AACTGTGATG TCGGTATGCG TAATTAAGTC CTCTACCGGA ACTTAACCA	
31601	TCACTTAATG CAGCAACAC AATTCCTCTC AAAAGAAAA TTGTGCAAGG CCTAGAAATT GATTCACACA AGCTATGCT TCCAAACTA GGAAGTGT	
	ATGTGATTAC GTGTGTTGTG TTGAGGGGAG TTTTGTGTTT AACCGTACC GATCTTAAA CTATGTTTGT TCCGATAGCA AGGATTTGAT CCTTACCTG	
31701	TTATTTTGA CAGCAGAGT GCGATTTGAG TAGGAAACAA AATATAGAT AAGCTAATTT TTGTGACCC ACCAGTCCA TCTGCTTACT CTGACTTAA	
	AATCAAACT GTGTGTGCA CCGTAAATGC ATCTTTGTT TTATTTCTA TTGATTTGA ACACCTGAG TGTGCTGAGT AGAGGATGTA CATCTGATTT	
31801	TGACAGGAAA GATGCTTAC TCACTTGT GTTACAAA TGTGCAATG AATATCTTC TACATTTCA GTTTTGTGCT TTAAAGGCGG TTGCTGCTCA	
	ACGTCTCTTT TACGATTT AGTGAACCA GATTTGTTT ACACGCTAG TTATGTAGAG ATGTCAATGT CAAGACCGAC AATTTGCTTC AAGCGAGT	
31901	ATATCTGAAA CAGTTGAGG TGTCAATCT ATATAGAT TTGCAAAA TTGATGCTA CTAAACAAT CTCTCTGGA CCAATATAT T. AACTTTA	
	TATAGACTT GTCAAGTTT ACAGTAGA TATATTTA AACTGTTT ACCTACGAT CATTTGTTAA GBAAGGAGCT GGTCTTATA ACCTGAAAT	
	Bgl	
32001	GAATGAGGA TCTTACTGAA GGCAGACTT ATACAAAGC TTGTGATTT ATGCTTAC TTATGACTTA TCCAAATCT CAGGTAAAA GTGCCAAGG	
	CTTTACCTCT AGATGACTT CCGTGTGGA TATGTTTGG ACACCTAAA TACTGATTTG ATAGTGAAT AGGTTTTGA GTGCCATTTT GAGCTTTTC	

Figure 15T

## pHRA15999g MFR612

32101 TACATTTGTC AGTCAAGTTT ACTTAAGGAG AGCAAAAGAT AAACCTGTMA CACTAACAT TACACTAAC GGTACACAG ANACAGAGA CACAACTCA  
 ATTGTACAG TCAGTTTCA TCAATTTGTC TCAATTTTCA TTCTACAT TCTATTTGTA ATGTATTTG CCAATGTGTC TTGTGCTCT GTGTGTAGT  
 32201 AGTGCATCT CTATGTCAT TTCAATGAGC TTCTATGAGT ACATACAT TATTAATATA TTGTACAT TTGTACAT CACTACAC CACTACAC ATTGCCAN  
 TCACATATGA GATACAGTAA AGTACCTG AGTACAGTAA TTCTATGAGT ATCTATTTAT AAACCTGTMA GGTAAATGTC AAAGATGAT TACTGTCTT  
 32301 AATAAGAAAT CATTGTGTT ATGTTTCAC GATTATTTT TTCAATTTTA GAUATTTTA ATCTATTTT CATTACAGT TATAGCCCA CCAATACATA  
 TTATTTCTTA GCAACACAA TACAAAGTTC CACAATATA AGTTTAAGT CTTTAAAGT TCACTAATA GTAGTCAAT ATATGCTGT TGTGTGTGTA  
 32401 GCTTATACAG ATCAAGCTAC CTTAATCMA CTCACAGAC CTTAGTATTC AACTGTAC TTCTATGAGT CTTGTGCTC TACACAGTCC TTCTGTCTT  
 GAAATATGTC TACTGTGAT GAAATATTT GAAATATTTT GAAATATTTT GAAATATTTT TCTATGAGT TCTATGAGT TCTATGAGT TCTATGAGT  
 32501 GCTGTGCTTA AAAGCATCA TATCATGCT TATCATGCT TATCATGCT TATCATGCT TATCATGCT TATCATGCT TATCATGCT TATCATGCT  
 GCAAGCAAT TTCTGTGAT ATATGCTGAT ATATGCTGAT ATATGCTGAT ATATGCTGAT ATATGCTGAT ATATGCTGAT ATATGCTGAT  
 32601 ATAAACTGTC GCGGAGCTC ACTTAAGTTC ATGTGCTGAT ATGTGCTGAT ATGTGCTGAT ATGTGCTGAT ATGTGCTGAT ATGTGCTGAT  
 TATTTGAGG GCGGCTGAG TAAATGAG TAAATGAG TAAATGAG TAAATGAG TAAATGAG TAAATGAG TAAATGAG TAAATGAG  
 32701 AAGTCCAGC CTACATGAG GTAGATGAT ATGTGCTGAT ATGTGCTGAT ATGTGCTGAT ATGTGCTGAT ATGTGCTGAT ATGTGCTGAT  
 TCAAGTGG GATGTACGTC CATCTCAGTA TTAGCAGTAA GTCTATGTC GTCACAGCA CTTGTGCTGAT CACTATTTG ACAGAGGAG CCGGAGGAG  
 32801 CCGTCCAGTA TACAGAGG CAGTGTGTC CTGAGGATG ATGTGCTGAT ATGTGCTGAT ATGTGCTGAT ATGTGCTGAT ATGTGCTGAT  
 GCACTGCTT ATGTGTACC GTACAGGAG GAGTGTGAT TAAGCTGAT TAAGCTGAT TAAGCTGAT TAAGCTGAT TAAGCTGAT  
 32901 TCACTTAAT TACAGAGTA ACTGAGCAG ACAGAGCAG TATTTTCTA TATTTTCTA TATTTTCTA TATTTTCTA TATTTTCTA TATTTTCTA  
 AGTGAATTTA GTGTGTGAT TCACTGCTG TCACTGCTG TCACTGCTG TCACTGCTG TCACTGCTG TCACTGCTG TCACTGCTG  
 33001 AACCACTG GCACTGAT CACAGAGTA GGTAGATTA GTGTGCTG GTGTGCTG GTGTGCTG GTGTGCTG GTGTGCTG GTGTGCTG  
 TTGTGTGAG CCACTGATG GTGTGCTG CCACTGAT CCACTGAT CCACTGAT CCACTGAT CCACTGAT CCACTGAT CCACTGAT  
 33101 CAGCACTG CCACTGAT TAACTGAT ATTAAGAT GCGCAGTA CCACTGAT CCACTGAT CCACTGAT CCACTGAT CCACTGAT CCACTGAT  
 GTGTGTGAG CCACTGAT ATTTGAGT TATTTGAT CCACTGAT CCACTGAT CCACTGAT CCACTGAT CCACTGAT CCACTGAT  
 33201 AGCAAGCAG GACTGAGTA ATGAGAGT AGAGGCTG CCACTGAT CCACTGAT CCACTGAT CCACTGAT CCACTGAT CCACTGAT  
 TCCCTGTG CCACTGAT TACTGTG CCACTGAT CCACTGAT CCACTGAT CCACTGAT CCACTGAT CCACTGAT CCACTGAT  
 33301 CCACTGAT CCACTGAT TCACTGAT CCACTGAT CCACTGAT CCACTGAT CCACTGAT CCACTGAT CCACTGAT CCACTGAT  
 CCACTGAT CCACTGAT TCACTGAT CCACTGAT CCACTGAT CCACTGAT CCACTGAT CCACTGAT CCACTGAT CCACTGAT  
 33401 AGCACTG CCACTGAT CCACTGAT CCACTGAT CCACTGAT CCACTGAT CCACTGAT CCACTGAT CCACTGAT CCACTGAT  
 TCACTGAT CCACTGAT CCACTGAT CCACTGAT CCACTGAT CCACTGAT CCACTGAT CCACTGAT CCACTGAT  
 33501 CCACTGAT CCACTGAT CCACTGAT CCACTGAT CCACTGAT CCACTGAT CCACTGAT CCACTGAT CCACTGAT CCACTGAT  
 CCACTGAT CCACTGAT CCACTGAT CCACTGAT CCACTGAT CCACTGAT CCACTGAT CCACTGAT CCACTGAT

Figure 15U





## pMRKarl5gag M2R6R2

35301 CATTATGAG AACTACAAAT TCCACACAA TACAAATTAC TCCCTCTAA AACTAGGTC ACCCTGCTCCG TTCCCAAGCC TTCCCAAGCC CCCCCTGACG TCACAAACTC  
GTAAATTTCT TTGATGTTTA AGGTTTGCT ATGTTCAATG ACCCTGCTCCG TTGATGAGGTC TTGATGAGGTC TTGATGAGGTC TTGATGAGGTC TTGATGAGGTC

35401 CACCCCTCA TTATCAATTT GCTTCAATC CAATATAGG TATATATAG TATATATAG TATATATAG TATATATAG TATATATAG TATATATAG TATATATAG  
GTGGGAGT AATGATATTA CCAATTTAG GTTTTATTC ATATATATC TACTATATTT ATTTCTTAA GCTTATAGG CTTATAGGCT GCTTATAGG CTTATAGGCT

35501 CCAATATGA TTCTTCTGC TTCCGCGGC ATCGGATTC CCGTCTTGA ATCGGATTC CCGTCTTGA ATCGGATTC CCGTCTTGA ATCGGATTC CCGTCTTGA  
GGTATATCT AGAGAGAGG AGGCGCGCG TACGCTTAC GCGCAATCT CCGTCTTGA ATCGGATTC CCGTCTTGA ATCGGATTC CCGTCTTGA ATCGGATTC

35601 CCAACCAAA GGCACCAAC GTTAAAGG CCGCTTCTC GCGCAATCT CCGTCTTGA ATCGGATTC CCGTCTTGA ATCGGATTC CCGTCTTGA ATCGGATTC  
CGTCTTCTC CCGTCTTCTC CCGTCTTCTC CCGTCTTCTC CCGTCTTCTC CCGTCTTCTC CCGTCTTCTC CCGTCTTCTC CCGTCTTCTC CCGTCTTCTC

35701 GAGGTGCGA ACCCTACAG GACTATTAAG ATACAGAGG TATCGCTTC ATACAGAGG TATCGCTTC ATACAGAGG TATCGCTTC ATACAGAGG TATCGCTTC  
CTCAGCGCT TTGCTCTCTC GCGAGCGCT GCGCTTCTC CCGCAATCT CCGTCTTGA ATCGGATTC CCGTCTTGA ATCGGATTC CCGTCTTGA ATCGGATTC

35801 CTGCTCTCTC TTGCTCTCTC GCGAGCGCT GCGCTTCTC CCGCAATCT CCGTCTTGA ATCGGATTC CCGTCTTGA ATCGGATTC CCGTCTTGA ATCGGATTC  
GAGGCGCGA AGAGCGCGA CCGTCTCTC CCGTCTCTC CCGTCTCTC CCGTCTCTC CCGTCTCTC CCGTCTCTC CCGTCTCTC CCGTCTCTC

35901 TCCACCAAC CCGCTCTCTC CCGTCTCTC CCGTCTCTC CCGTCTCTC CCGTCTCTC CCGTCTCTC CCGTCTCTC CCGTCTCTC CCGTCTCTC  
ACGCTCTCTC CCGTCTCTC CCGTCTCTC CCGTCTCTC CCGTCTCTC CCGTCTCTC CCGTCTCTC CCGTCTCTC CCGTCTCTC CCGTCTCTC

36001 CACTGCTTAC AGATATGAG GAGCTCTCTC GAGCTCTCTC GAGCTCTCTC GAGCTCTCTC GAGCTCTCTC GAGCTCTCTC GAGCTCTCTC GAGCTCTCTC  
GTATACCAAT TCTATATCT CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC

36101 ATCTGCTCTC TCTATATCT CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC  
TACAGCGAG AGAGCTCTC TCTATATCT CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC

36201 AGCAGCAAT TACGCGCGA AATATATCT CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC  
TCTCTCTCT ATGCGCTCT TCTATATCT CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC

36301 TTGCTCTCTC AGATATCT AATATATCT CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC  
AAGCAGTAC TCTATATCT TTGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC

36401 TCAATGAGG ACCTATCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC  
AGTCACTCG TCGATATCT CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC

36501 TCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC  
ACGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC

36601 CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC  
GAGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC

36701 CTACAGCAT CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC  
GATCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC

36801 AAGCTCTCTC AGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC CCGCTCTCTC  
TTTCTCTCTC TCGAGAGG CAGAGAGG CAGAGAGG CAGAGAGG CAGAGAGG CAGAGAGG CAGAGAGG CAGAGAGG CAGAGAGG CAGAGAGG

36901 GTCATGCT CCGTATAT CCGTATAT CCGTATAT CCGTATAT CCGTATAT CCGTATAT CCGTATAT CCGTATAT CCGTATAT CCGTATAT  
CAGTATAT GGCATATCT GGCATATCT GGCATATCT GGCATATCT GGCATATCT GGCATATCT GGCATATCT GGCATATCT GGCATATCT GGCATATCT

Figure 15W



pMRKAd5q1g M5K682  
 37001 CAGACCGGGA TAAATCCCGG CCACATACCA GAACTTTTAA AGTATTCATC ATTGTAAAC GTTCTTGGG GCGAAACAC TCAGAGATCT TACTTCTTCT  
 GTTGTGCCCC ATTATGGGCG GTGTATATCT CTTCGAATTT TACGACATG TACCTTTTG CAGACGCC CCGTTTTCAG AGTTCCCTAGA ATGCGACAA  
 GAGATCCAGT TCGATGTAA CCACTCTGCG ACCCACTTA TCTTAATAT TCTTAACTT CACTACCTT TCTGCTTGA CAGAACAGG AATGCAAAAT  
 CTCATGCTCA AGCTACATG GTTCAGCAGG TGTGTGCACT AGAATTTTGA GAAATGTAA GTATCTGCA AGACCCACT GTTTTGTCC TCCGTTTAA  
 GCGCCAAAAA AGGGAATAG GCGGACACGG AATGTTGGA TATATATCT TTCTTTT CAAATATAT GAGCAATTA TCAGATTAAT AGTCCCAATA ACAGATTAAT  
 CCGCGTTTTT TCCCTATTC CCGCTGTGCT TTACAACTT ATGATATCA GAGCAAAAA GTTATATTA CTTCCATAAT AGTCCCAATA ACAGATTAAT  
 GCGGATACAT ATTGAAATGT ATTAGAAAA ATAAACAAAT AGGATTTG CCAATATTC CCGCAAAAT GCGACCTGAC GTCTAGAAA CCAATTATTA  
 CCGCTATGTA TAACTTACA TAAATCTTTT TATTGTGTTA TCCCAAGG CCGGTAAAG GAGCTTTCA CCGTGAAGT CAGATCTCTT CCAATAAATA  
 37101  
 37201  
 37301  
 37401 CATGACATTA ACCTATAA ATAGCGTAT CACGAGGCC TTCTGCTCTC AAGAAATGGA TTCCGATCT TAAAT (SEQ ID NO: 27)  
 GTACTGTAAT TGGATTTTTT TATCCCATTA GTCTCCCGG AAGCAGCAG TCTTAACTT AGCCTTAAAG ATTA (SEQ ID NO: 28)

Figure 15X

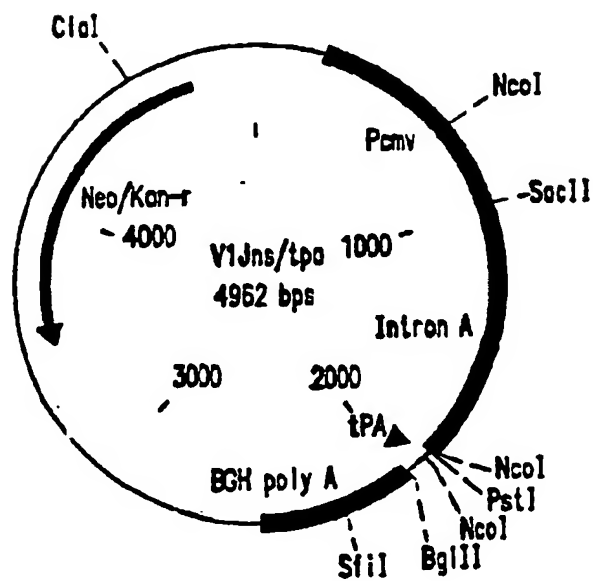
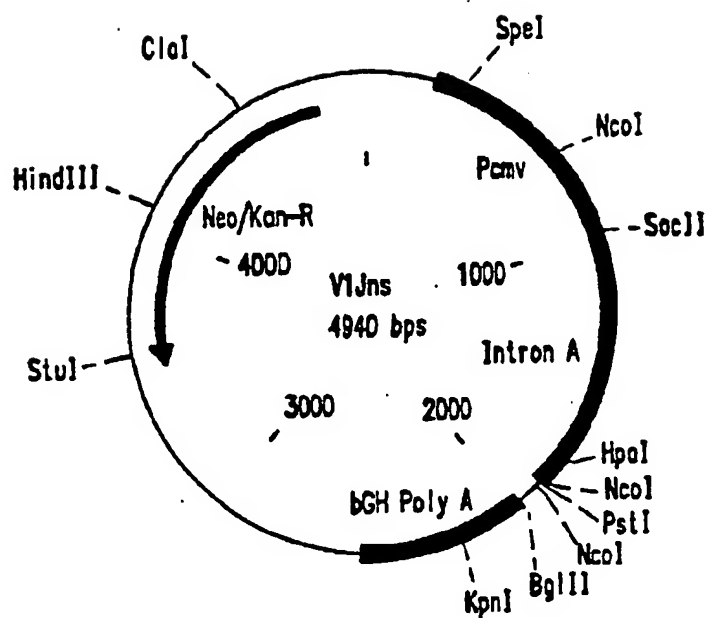


FIGURE 16

AGATCTACCATGGCCCCCATCTCCCCATTGAGACTGTGCTGTGAAGCTGAAGCCTGGCATGGATGGCCCCAAGTGAA  
 Bg/11 MetAlaProIleSerProIleGluThrValProValLysLeuLysProGlyMetAspGlyProLysValLys  
 1 10 20

GCAGTGGCCCTGACTGAGGAGAAGATCAAGGCCCTGGTGGAAATCTGCACTGAGATGGAGAAGGAGGGCAAAATCTCCA  
 sGlnTrpProLeuThrGluGluLysIleLysAlaLeuValGluIleCysThrGluMetGluLysGluGlyLysIleSerL  
 30 40 50

AGATTGGCCCGAGAACCCTACAACACCCTGTGTTTGCCATCAAGAAGAAGGACTCCACCAAGTGGAGGAACCTGGTG  
 ysIleGlyProGluAsnProTyrAsnThrProValPheAlaIleLysLysLysAspSerThrLysTrpArgLysLeuVal  
 60 70

GACTTCAGGGAGCTGAACAAGAGGACCCAGGACTTCTGGGAGGTGCAGCTGGGCATCCCCACCCCGCTGGCCTGAAGAA  
 AspPheArgGluLeuAsnLysArgThrGlnAspPheTrpGluValGlnLeuGlyIleProHisProAlaGlyLeuLysLys  
 80 90 100

GAAGAAGTCTGTGACTGTGCTGGCTGTGGGGATGCCTACTTCTGTGCCCCCTGGATGAGGACTTCAGGAAGTACACTG  
 sLysLysSerValThrValLeuAlaValGlyAspAlaTyrPheSerValProLeuAspGluAspPheArgLysTyrThrA  
 110 120 130

CCTTCACCATCCCTCCATCAACAATGAGACCCCTGGCATCAGGTACCAATGTGCTGCCCCAGGGCTGGAAGGGC  
 loPheTrnIleProSerIleAsnAsnGluThrProGlyIleArgTyrGlnTyrAsnValLeuProGlnGlyTrpLysGly  
 140 150

TCCCTGCCATCTTCCAGTCTCCATGACCAAGATCCTGGAGCCCTTCAGGAAGCAGAACCCTGACATTGTGATCTACCA  
 SerProAlaIlePheGlnSerSerMetThrLysIleLeuGluProPheArgLysGlnAsnProAspIleValIleTyrG  
 160 170 180

GTACATGGCTGCCCTGTATGTGGGCTCTGACCTGGAGATTGGGCAGCACAGGACCAAGATTGAGGAGCTGAGGCAGCACC  
 nTyrMetAlaAlaLeuTyrValGlySerAspLeuGluIleGlyGlnHisArgThrLysIleGluGluLeuArgGlnHisL  
 190 200 210

TGCTGAGGTGGGCTGACCAACCCCTGACAAGAAGCACCAGAAGGAGCCCCCTTCTGTGGATGGGCTATGAGCTGCAC  
 euLeuArgTrpGlyLeuThrThrProAspLysLysHisGlnLysGluProProPheLeuTrpMetGlyTyrGluLeuHis  
 220 230

CCGACAAGTGGACTGTGCAGCCCATTTGTGCTGCCTGACAAGGACTCCTGGACTGTGAATGACATCCAGAAGCTGGTGGG  
 ProAspLysTrpThrValGlnProIleValLeuProGluLysAspSerTrpThrValAsnAspIleGlnLysLeuValG  
 240 250 260

CAAGCTGAAGTGGGCTCCCAAATCTACCCCTGGCATCAAGGTGAGGCAGCTGTGCAAGCTGCTGAGGGGCACCAAGGCC  
 yLysLeuAsnTrpAlaSerGlnIleTyrProGlyIleLysValArgGlnLeuCysLysLeuLeuArgGlyThrLysAlaL  
 270 280 290

FIGURE 17A

TGACTGAGGTGATCCCCCTGACTGAGGAGGCTGAGCTGGAGCTGGCTGAGAACAGGGAGATCCTGAAGGACCTGTGCAT  
 EuThrGluValIleProLeuThrGluGluAlaGluLeuGluLeuAlaGluAsnArgGluIleLeuLysGluProValHis  
 300 310

GGGGTGACTATGACCCCTCCAAGGACCTGATTGCTGAGATCCAGAAGCAGGGCCAGGGCCAGTGGACCTACCAATCTA  
 GlyValTyrTyrAspProSerLysAspLeuIleAlaGluIleGlnLysGlnGlyGlnGlyGlnTrpThrTyrGlnIleTy  
 320 330 340

CCAGGAGCCCTTCAAGAACCTGAAGACTGGCAAGTATGCCAGGATGAGGGGGCCACACCAATGATGTGAAGCAGCTGA  
 rGlnGluProPheLysAsnLeuLysThrGlyLysTyrAlaArgMetArgGlyAlaHisThrAsnAspValLysGlnLeuT  
 350 360 370

CTGAGGCTGTGCAGAAGATCACCAGTGAAGTCCATTGTGATCTGGGGCAAGACCCCAAGTTCAAGCTGCCATCCAGAAG  
 hrGluAlaValGlnLysIleThrThrGluSerIleValIleTrpGlyLysThrProLysPheLysLeuProIleGlnLys  
 380 390

GAGACCTGGGAGACCTGGTGGACTGAGTACTGGCAGGCCACCTGGATCCCTGAGTGGGAGTTTGTGAACACCCCCCCT  
 GluThrTrpGluThrTrpTrpThrGluTyrTrpGlnAlaThrTrpIleProGluTrpGluPheValAsnThrProProLe  
 400 410 420

GGTAAGCTGTGGTACCAGCTGGAGAAGGAGCCCATGTGGGGCTGAGACCTTCTATGTGGCTGGGGCTGCCAACAGGG  
 uValLysLeuTrpTyrGlnLeuGluLysGluProIleValGlyAlaGluThrPheTyrValAlaGlyAlaAlaAsnArgG  
 430 440 450

AGACCAAGCTGGGCAAGGCTGGCTATGTGACCAACAGGGCAGGCAGAAGGTGGTGACCTGACTGACACCACCAACCAG  
 luThrLysLeuGlyLysAlaGlyTyrValThrAsnArgGlyArgGlnLysValValThrLeuThrAspThrThrAsnGln  
 460 470

AAGACTGCCCTCCAGGCCATCTACCTGGCCCTCCAGGACTCTGGCCCTGGAGGTGAACATTGTGACTGCCCTCCAGTATGC  
 LysThrAlaLeuGlnAlaIleTyrLeuAlaLeuGlnAspSerGlyLeuGluValAsnIleValThrAlaSerGlnTyrAl  
 480 490 500

CCTGGGCATCATCCAGGCCAGCCTGATCAGTCTGAGTCTGAGCTGGTGAACCAGATCATTGAGCAGCTGATCAAGAAGG  
 dLeuGlyIleIleGlnAlaGlnProAspGlnSerGluSerGluLeuValAsnGlnIleIleGluGlnLeuIleLysLysG  
 510 520 530

AGAAGGTGTACCTGGCCTGGGTGCCCTGCCACAAGGGCATTGGGGCAATGAGCAGGTGGACAAGCTGGTGTCTGCTGGC  
 luLysValTyrLeuAlaTrpValProAlaHisLysGlyIleGlyGlyAsnGluGlnValAspLysLeuValSerAlaGly  
 540 550

ATCAGGAAGGTGCTGTTCTGGATGGCATTGACAAGGCCAGGATGAGCATGAGAAGTACCACTCCAACCTGGAGGGCTAT  
 IleArgLysValLeuPheLeuAspGlyIleAspLysAlaGlnAspGluHisGluLysTyrHisSerAsnTrpArgAlaMet  
 560 570 580

FIGURE 17B

GGCCTCTGACTTCAACCTGCCCCCTGTGGTGGCTAAGGAGATTGTGGCTCCTGTGACAAGTCCAGCTGAAGGGGAGG  
 tAlaSerAspPheAsnLeuProProValVolAlaLysGluIleVolAlaSerCysAspLysCysGlnLeuLysGlyGluA  
 590 600 610

CCATGCATGGGCAGGTGGACTGCTCCCTGGCATCTGGCAGCTGGCTGCACCCACCTGGAGGGCAAGGTGATCCTGGT  
 lAlaMetHisGlyGlnVolAspCysSerProGlyIleTrpGlnLeuAlaCysThrHisLeuGluGlyLysVolIleLeuVol  
 620 630

GCTGTGCATGTGGCTCCGGCTACATTGAGGCTGAGGTGATCCCTGCTGAGACAGGCCAGGAGACTGCCTACTTCCCTGCT  
 AlaValHisVolAlaSerGlyTyrIleGluAlaGluValIleProAlaGluThrGlyGlnGluThrAlaTyrPheLeuLe  
 640 650 660

GAAGCTGGCTGGCAGGTGGCTGTGAAGACCATCCACACTGCCAATGGCTCCAATTCACTGGGGCCACAGTGAGGGCTG  
 uLysLeuAlaGlyArgTrpProVolLysThrIleHisThrAlaAsnGlySerAsnPheThrGlyAlaThrVolArgAlaA  
 670 680 690

CCTGCTGGTGGGCTGGCATCAAGCAGGAGTTTGGCATCCCTACAACCCCCAGTCCAGGGGGTGGTGGCTCCATGAAC  
 lAlaCysTrpTrpAlaGlyIleLysGlnGluPheGlyIleProTyrAsnProGlnSerGlnGlyVolVolAlaSerMetAsn  
 700 710

AAGGAGCTGAAGAAGATCATTGGGCAGGTGAGGGACCGCTGAGCACCTGAAGACAGCTGTGCAGATGGCTGTGTTCAT  
 LysGluLeuLysLysIleIleGlyGlnVolArgAspGlnAlaGluHisLeuLysThrAlaVolGlnMetAlaVolPheIle  
 720 730 740

CCACAATTCAAGAGGAAGGGGGCATCGGGGCTACTCCGCTGGGAGAGGATTGTGGACATCATTGCCACAGACATCC  
 eHisAsnPheLysArgLysGlyGlyIleGlyGlyTyrSerAlaGlyGluArgIleVolAspIleIleAlaThrAspIleG  
 750 760 770

AGACCAAGGAGCTCCAGAAGCAGATCACCAAGATCCAGAATTCAAGGTGTACTACAGGAGCTCCAGGAACCCCTGTGG  
 lnThrLysGluLeuGlnLysGlnIleThrLysIleGlnAsnPheArgValTyrTyrArgAspSerArgAsnProLeuTrp  
 780 790

AAGGGCCCTGCCAAGCTGCTGTGGAAGGGGAGGGGGCTGTGGTGTATCCAGGACAACCTCTGACATCAAGGTGGTGGCCAG  
 LysGlyProAlaLysLeuLeuTrpLysGlyGluGlyAlaVolVolIleGlnAspAsnSerAspIleLysVolVolProAr  
 800 810 820

GAGGAAGGCCAAGATCATCAGGACTATGGCAAGCAGATGGCTGGGATGACTGTGTGGCTCCAGGCAGGATGAGGACT  
 gArgLysAlaLysIleIleArgAspTyrGlyLysGlnMetAlaGlyAspAspCysValAlaSerArgGlnAspGluAspx  
 830 840 850

AAAGCCCCGGGCAGATC; (SEQ ID NO: 3)  
 Xx BgπI (SEQ ID NO: 4)

FIGURE 17C



WT	- ATG GGT GGC AAG TGG TCA AAA CGT AGT GTG CCT GGA TGG TCT	-42
OPT	- ATG GGC GGC AAG TGG TCC AAG AGG TCC GTG CCC GGC TGG TCC	
	M G G K W S K R S V P G W S	-14
WT	- ACT GTA AGG GAA AGA ATG AGA CGA GCT GAG CCA GCA GCA GAT	-84
OPT	- ACC GTG AGG GAG AGG ATG AGG AGG GCC GAG CCC GCC GCC GAC	
	T V R E R M R R A E P A A D	-28
WT	- AGG GTG AGA CGA ACT GAG CCA GCA GCA GTA GGG GTG GGA GCA	-126
OPT	- AGG GTG AGG AGG ACC GAG CCC GCC GCC GTG GGC GTG GGC GCC	
	R V R R T E P A A V G V G A	-42
WT	- GTA TCT CGA GAC CTG GAA AAA CAT GGA GCA ATC ACA AGT AGC	-168
OPT	- GTG TCC AGG GAC CTG GAG AAG CAC GGC GCC ATC ACC TCC TCC	
	V S R D L E K H G A I T S S	-56
WT	- AAT ACA GCA GCT ACC AAT GCT GAT TGT GCC TGG CTA GAA GCA	-210
OPT	- AAC ACC GCC GCC ACC AAC GCC GAC TGC GCC TGG CTG GAG GCC	
	N T A A T N A D C A W L E A	-70
WT	- CAA GAG GAT GAG GAA GTG GGT TTT CCA GTC AGA CCT CAG GTA	-252
OPT	- CAG GAG GAC GAG GAG GTG GGC TTC CCC GTG AGG CCC CAG GTG	
	Q E D E E V G F P V R P Q V	-84
WT	- CCT TTA AGA CCA ATG ACT TAC AAG GGA GCT GTA GAT CTT AGC	-294
OPT	- CCC CTG AGG CCC ATG ACC TAC AAG GGC GCC GTG GAC CTG TCC	
	P L R P M T Y K G A V D L S	-98
WT	- CAC TTT TTA AAA GAA AAG GGG GGA CTG GAA GGG CTA ATT CAC	-336
OPT	- CAC TTC CTG AAG GAG AAG GGC GGC CTG GAG GGC CTG ATC CAC	
	H F L K E K G G L E G L I H	-112
WT	- TCA CAG AAA AGA CAA GAT ATC CTT GAT CTG TGG GTC TAC CAC	-378
OPT	- TCC CAG AAG AGG CAG GAC ATC CTG GAC CTG TGG GTG TAC CAC	
	S Q K R Q D I L D L W V Y H	-126
WT	- ACA CAA GGC TAC TTC CCT GAT TGG CAG AAC TAC ACA CCA GGG	-420
OPT	- ACC CAG GGC TAC TTC CCC GAC TGG CAG AAC TAC ACC CCC GGC	
	T Q G Y F P D W Q N Y T P G	-140

FIGURE 19A

WT	- CCA GGA ATC AGA TTT CCA TTG ACC TTT GGA TGG TGC TTC AAG	-462
OPT	- CCC GGC ATC AGG TTC CCC CTG ACC TTC GGC TGG TGC TTC AAG	
	P G I R F P L T F G W C F K	-154
WT	- CTA GTA CCA GTT GAG CCA GAA AAG GTA GAA GAG GCC AAT GAA	-504
OPT	- CTG GTG CCC GTG GAG CCC GAG AAG GTG GAG GAG GCC AAC GAG	
	L V P V E P E K V E E A N E	-168
WT	- GGA GAG AAC AAC TGC TTG TTA CAC CCT ATG AGC CAG CAT GGG	-546
OPT	- GGC GAG AAC AAC TGC CTG CTG CAC CCC ATG TCC CAG CAC GGC	
	G E N N C L L H P M S Q H G	-182
WT	- ATA GAG GAC CCG GAG AAG GAA GTG TTA GAG TGG AGG TTT GAC	-588
OPT	- ATC GAG GAC CCC GAG AAG GAG GTG CTG GAG TGG AGG TTC GAC	
	I E D P E K E V L E W R F D	-196
WT	- AGC AAG CTA GCA TTT CAT CAC GTG GCC CGA GAG CTG CAT CCG	-630
OPT	- TCC AAG CTG GCC TTC CAC CAC GTG GCC AGG GAG CTG CAC CCC	
	S K L A F H H V A R E L H P	-210
WT	- GAG TAC TAC AAG GAC TGC TGA (SEQ ID NO:30)	-651
OPT	- GAG TAC TAC AAG GAC TGC TAA (contained within SEQ ID NO:9)	
	E Y Y K D C (SEQ ID NO:10)	-216

FIGURE 19B



## FIGURE 20

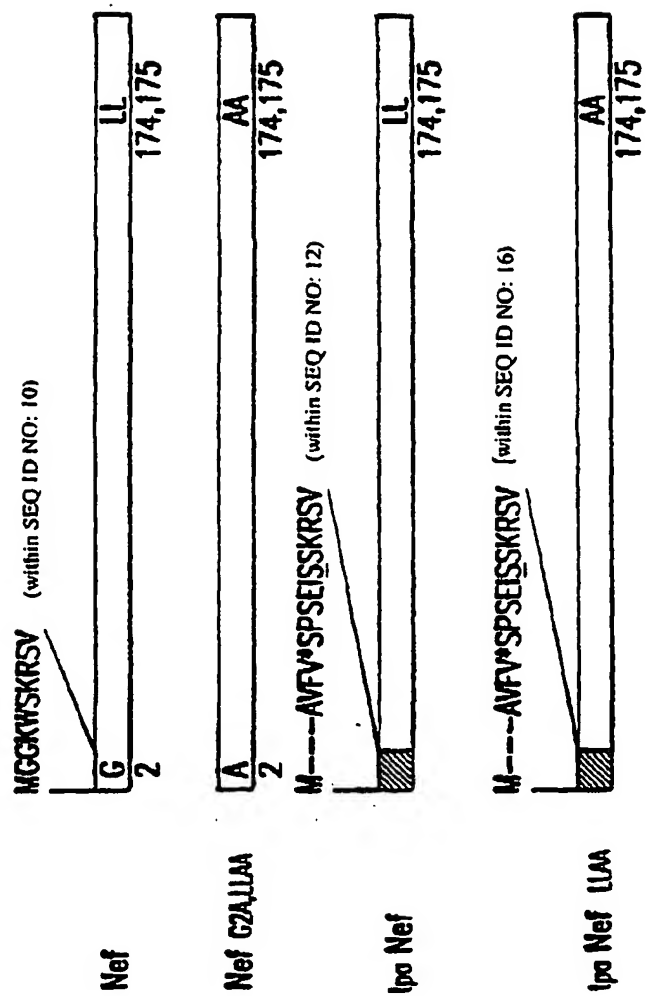


FIGURE 21

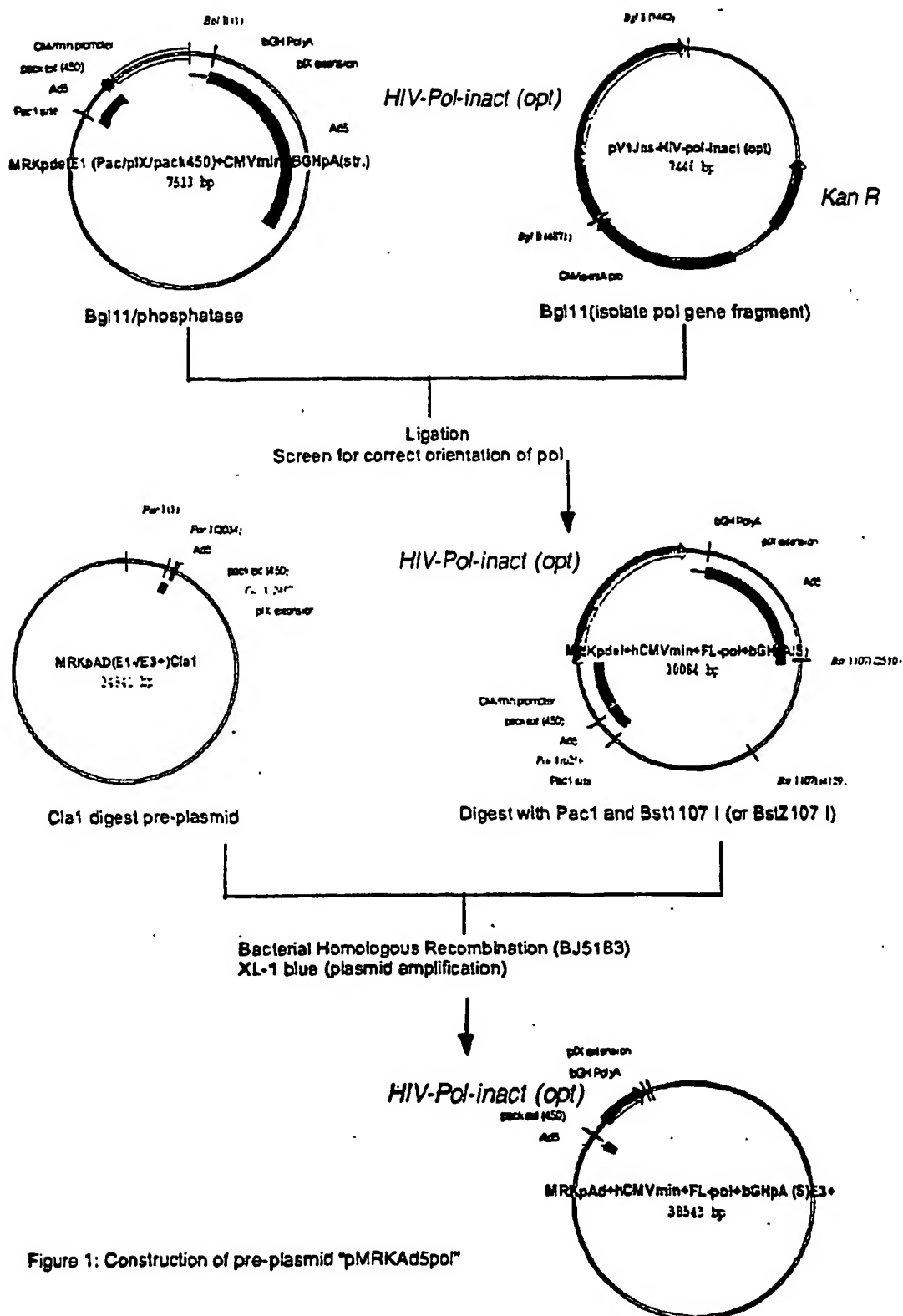
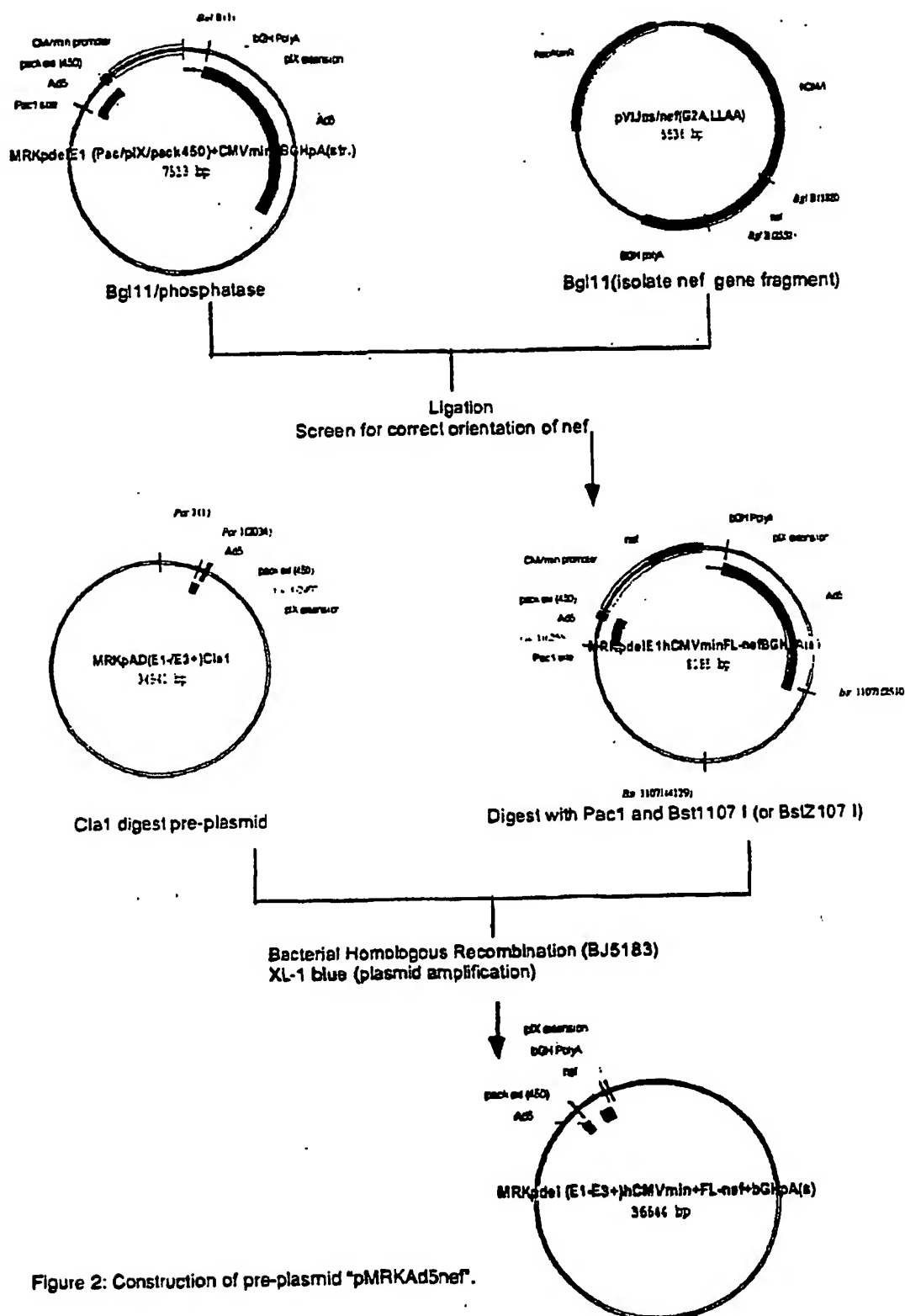


Figure 1: Construction of pre-plasmid "pMRKAd5pol"

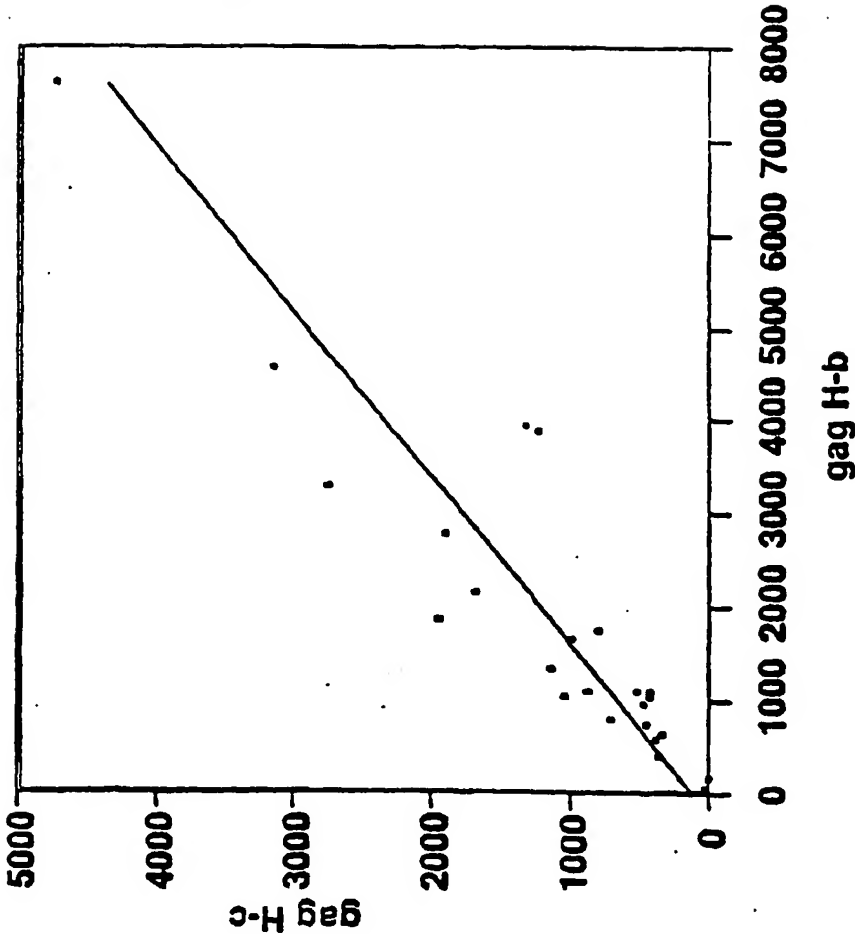
FIGURE 22



**Figure 2: Construction of pre-plasmid “pMRKAd5nef”.**

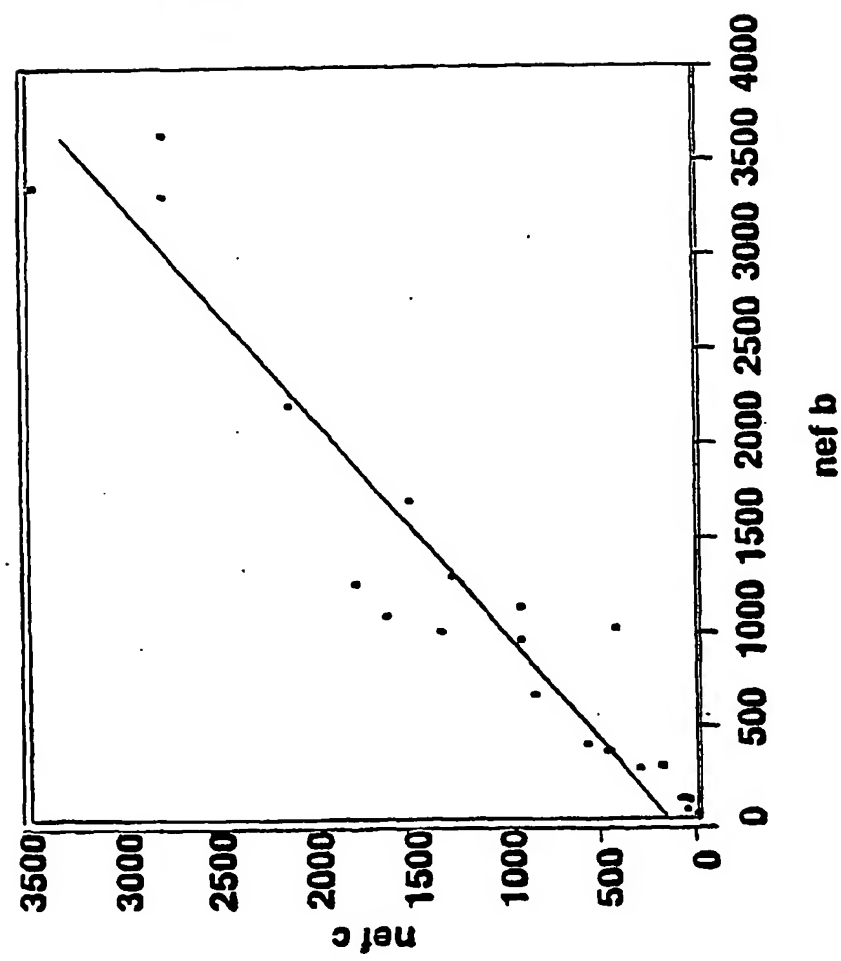
**FIGURE 23**

**Comparison of Clade B vs. Clade C Anti-gag T Cell Responses in Clade B HIV-Infected Subjects**



Linear Fit	
gag H-c = 111.603 + 0.55866 gag H-b	
Summary of Fit	
RSquare	0.816775
RSquare Adj	0.80914
Root Mean Square Error	474.9639
Mean of Response	1158.115
Observations (or Sum Wgts)	26

Comparison of Clade B vs. Clade C Anti-nef T Cell Responses in Clade B HIV-Infected Subjects



nef c = 131.132 + 0.8646 nef b

Summary of Fit	
RSquare	0.91685
RSquare Adj	0.91289
Root Mean Square Error	289.7718
Mean of Response	1096.435
Observations (or Sum Wgts)	23

FIGURE 25

**MRKAd5pol MER1062**  
**(MRKAd5 Pre-Adenoviral Vector Containing the LA opt pol Coding Region)**

```

1  CATCATCAAT AATATACCTT ATTTTGGATT GAAGCCAATA TGATAATGAG
   GTAGTAGTTA TTATATGGAA TAAACCTAA CTTCGGTTAT ACTATTACTC

51  GGGGTGGAGT TTGTGACGTG GCGCGGGGCG TGGGAACGGG GCGGGTGACG
   CCCACCTCA AACACTGCAC CGCGCCCCGC ACCCTTGCCC CGCCCACTGC

101 TAGTAGTGTG GCGGAAGTGT GATGTTGCAA GTGTGGCGGA ACACATGTAA
   ATCATCACAC CGCCTTCACA CTACAACGTT CACACCGCCT TGTGTACATT

151 GCGACGGATG TGGCAAAAGT GACGTTTTTG GTGTGCGCCG GTGTACACAG
   CGCTGCCTAC ACCGTTTTCA CTGCAAAAC CACACCGCGC CACATGTGTC

201 GAAGTGACAA TTTTCGCGCG GTTTTAGGCG GATGTTGTAG TAAATTTGGG
   CTTCACTGTT AAAAGCGCGC CAAATCCGC CTACAACATC ATTTAAACCC

251 CGTAACCGAG TAAGATTTGG CCATTTTCGC GGGAAACTG AATAAGAGGA
   GCATTGGCTC ATTCTAAACC GGTAAAAGCG CCCTTTTGAC TTATTCTCCT

301 AGTGAAATCT GAATAATTTT GTGTTACTCA TAGCGCGTAA TATTTGTCTA
   TCACCTTTAGA CTTATTAATA CACAATGAGT ATCGCGCATT ATAAACAGAT

351 GGGCCGCGGG GACTTTGACC GTTTACGTGG AGACTCGCCC AGGTGTTTTT
   CCCGGCGCCC CTGAAACTGG CAAATGCACC TCTGAGCGGG TCCACAAAAA

401 CTCAGGTGTT TTCCGCGTTC CGGGTCAAAG TTGGCGTTTT ATTATTATAG
   GAGTCCACAA AAGGCGCAAG GCCCAGTTTC AACCACAAA TAATAATATC

451 GCGGCCGCGA TCCATTGCAT ACGTTGTATC CATATCATAA TATGTACATT
   CGCCGGCGCT AGGTAACGTA TGCAACATAG GTATAGTATT ATACATGTAA

501 TATATTGGCT CATGTCCAAC ATTACCGCCA TGTTGACATT GATTATTGAC
   ATATAACCGA GTACAGGTTG TAATGGCGGT ACAACTGTAA CTAATAACTG

551 TAGTTATTAA TAGTAATCAA TTACGGGGTC ATTAGTTCAT AGCCCATATA
   ATCAATAATT ATCATTAGTT AATGCCCCAG TAATCAAGTA TCGGGTATAT

601 TGGAGTTCCG CGTTACATAA CTTACGTAA ATGGCCCGCC TGGCTGACCG
   ACCTCAAGGC GCAATGTATT GAATGCCATT TACCGGGCGG ACCGACTGGC

651 CCCAACGACC CCCGCCAAT GACGTCAATA ATGACGTATG TTCCCATAGT
   GGGTTGCTGG GGGCGGGTAA CTGCAGTTAT TACTGCATAC AAGGGTATCA

701 AACGCCAATA GGGACTTTCC ATTGACGTCA ATGGGTGGAG TATTTACGGT
   TTGCGGTAT CCCTGAAAGG TAACTGCAGT TACCCACCTC ATAAATGCCA

751 AAAGTGGCCA CTTGGCAGTA CATCAAGTGT ATCATATGCC AAGTACGCCC
   TTTGACGGGT GAACCGTCAT GTAGTTCACA TAGTATACGG TTCATGCGGG

801 CCTATTGACG TCAATGACGG TAAATGGCCC GCCTGGCATT ATGCCAGTA
   GGATAACTGC AGTTACTGCC ATTTACCGGG CGGACCGTAA TACGGGTCAT

851 CATGACCTTA TGGGACTTTC CTACTGGCA GTACATCTAC GTATTAGTCA
   GTACTGGAAT ACCCTGAAAG GATGAACCGT CATGTAGATG CATAATCAGT

```

*Figure 26A*

901 TCGCTATTAC CATGGTGATG CGGTTTTGGC AGTACATCAA TGGGCGTGGA  
AGCGATAATG GTACCACTAC GCCAAAACCG TCATGTAGTT ACCCGCACCT

951 TAGCGGTTTG ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA  
ATCGCCAAAC TGAGTGCCCC TAAAGGTTCA GAGGTGGGGT AACTGCAGTT

1001 TGGGAGTTTG TTTTGGCACC AAAATCAACG GGACTTTCCA AAATGTCGTA  
ACCCCTCAAAC AAAACCGTGG TTTTAGTTGC CCTGAAAGGT TTTACAGCAT

1051 ACAACTCCGC CCCATTGACG CAAATGGGCG GTAGGCGTGT ACGGTGGGAG  
TGTTGAGGCG GGGTAACTGC GTTTACCCGC CATCCGCACA TGCCACCCTC

1101 GTCTATATAA GCAGAGCTCG TTTAGTGAAC CGTCAGATCG CCTGGAGACG  
CAGATATATT CGTCTCGAGC AAATCACTTG GCAGTCTAGC GGACCTCTGC

1151 CCATCCACGC TGTTTTGACC TCCATAGAAG ACACCGGGAC CGATCCAGCC  
GGTAGGTGCG ACAAACCTGG AGGTATCTTC TGTGGCCCTG GCTAGGTGCG

1201 TCCGCGGCCG GGAACGGTGC ATTGGAACGC GGATTCCCCG TGCCAAGAGT  
AGGCGCCGGC CCTTGCCACG TAACCTTGCG CCTAAGGGGC ACGGTTCTCA

1251 GAGATCTACC ATGGCCCCCA TCTCCCCCAT TGAGACTGTG CCTGTGAAGC  
CTCTAGATGG TACCGGGGGT AGAGGGGGTA ACTCTGACAC GGACACTTCG

1301 TGAAGCCTGG CATGGATGGC CCCAAGGTGA AGCAGTGGCC CCTGACTGAG  
ACTTCGGACC GTACCTACCG GGGTTCCACT TCGTCACCGG GGACTGACTC

1351 GAGAAGATCA AGGCCCTGGT GGAAATCTGC ACTGAGATGG AGAAGGAGGG  
CTCTTCTAGT TCCGGGACCA CCTTTAGACG TGACTCTACC TCTTCCTCCC

1401 CAAATCTCC AAGATTGGCC CCGAGAACCC CTACAACACC CCTGTGTTTG  
GTTTTAGAGG TTCTAACCGG GGCTCTTGGG GATGTTGTGG GGACACAAAC

1451 CCATCAAGAA GAAGGACTCC ACCAAGTGGA GGAAGCTGGT GGACTTCAGG  
GGTAGTTCTT CTTCTGAGG TGGTTCACCT CCTTCGACCA CCTGAAGTCC

1501 GAGCTGAACA AGAGGACCCA GGACTTCTGG GAGGTGCAGC TGGGCATCCC  
CTCGACTTGT TCTCCTGGGT CCTGAAGACC CTCCACGTCG ACCCGTAGGG

1551 CCACCCCGCT GGCCTGAAGA AGAAGAAGTC TGTGACTGTG CTGGCTGTGG  
GGTGGGGCGA CCGGACTTCT TCTTCTTCAG AACTTGACAC GACCGACAC

1601 GGGATGCCTA CTTCTCTGTG CCCCTGGATG AGGACTTCAG GAAGTACACT  
CCCTACGGAT GAAGAGACAC GGGGACCTAC TCCTGAAGTC CTTTCATGTGA

1651 GCCTTCACCA TCCCCTCCAT CAACAATGAG ACCCCTGGCA TCAGGTACCA  
CGGAAGTGGT AGGGGAGGTA GTTGTTACTC TGGGGACCGT AGTCCATGGT

1701 GTACAATGTG CTGCCCCAGG GCTGGAAGGG CTCCCCTGCC ATCTTCCAGT  
CATGTTACAC GACGGGGTCC CGACCTTCCC GAGGGGACGG TAGAAGGTCA

1751 CCTCCATGAC CAAGATCCTG GAGCCCTTCA GGAAGCAGAA CCCTGACATT  
GGAGGTACTG GTTCTAGGAC CTCGGGAAGT CCTTCGTCTT GGGACTGTAA

1801 GTGATCTACC AGTACATGGC TGCCCTGTAT GTGGGCTCTG ACCTGGAGAT  
CACTAGATGG TCATGTACCG ACGGGACATA CACCCGAGAC TGGACCTCTA



1901 GGGGCCTGAC CACCCCTGAC AAGAAGCACC AGAAGGAGCC CCCCTTCCTG  
 CCCC GGACTG GTGGGGACTG TTCTTCGTGG TCTTCCTCGG GGGGAAGGAC  
 1951 TGGATGGGCT ATGAGCTGCA CCCC GACAAG TGGACTGTGC AGCCCATTGT  
 ACCTACCCGA TACTCGACGT GGGGCTGTTC ACCTGACACG TCGGGTAACA  
 2001 GCTGCCTGAG AAGGACTCCT GGACTGTGAA TGACATCCAG AAGCTGGTGG  
 CGACGGACTC TTCCTGAGGA CCTGACACTT ACTGTAGGTC TTCGACCACC  
 2051 GCAAGCTGAA CTGGGCCTCC CAAATCTACC CTGGCATCAA GGTGAGGCAG  
 CGTTCGACTT GACCCGGAGG GTTTAGATGG GACCGTAGTT CCACTCCGTC  
 2101 CTGTGCAAGC TGCTGAGGGG CACCAAGGCC CTGACTGAGG TGATCCCCCT  
 GACACGTTTC ACGACTCCCC GTGGTTCCTGG GACTGACTCC ACTAGGGGGA  
 2151 GACTGAGGAG GCTGAGCTGG AGCTGGCTGA GAACAGGGAG ATCCTGAAGG  
 CTGACTCCTC CGACTCGACC TCGACCGACT CTTGTCCCTC TAGGACTTCC  
 2201 AGCCTGTGCA TGGGGGTGAC TATGACCCCT CCAAGGACCT GATTGCTGAG  
 TCGGACACGT ACCCCACATG ATACTGGGGA GGTTCTTGA CTAACGACTC  
 2251 ATCCAGAAGC AGGGCCAGGG CCAGTGGACC TACCAAATCT ACCAGGAGCC  
 TAGGTCTTCG TCCCGGTCCC GGTCACCTGG ATGGTTTAGA TGGTCTCTCG  
 2301 CTTCAAGAAC CTGAAGACTG GCAAGTATGC CAGGATGAGG GGGGCCACAC  
 GAAGTTCTTG GACTTCTGAC CGTTCATACG GTCCTACTCC CCCC GGSTGT  
 2351 CCAATGATGT GAAGCAGCTG ACTGAGGCTG TGCAGAAGAT CACCACTGAG  
 GGTTACTACA CTTCGTCGAC TGACTCCGAC ACGTCTTCTA GTGGTGACTC  
 2401 TCCATTGTGA TCTGGGGCAA GACCCCAAG TTCAAGCTGC CCATCCAGAA  
 AGGTAACACT AGACCCCGTT CTGGGGGTTC AAGTTCGACG GGTAGGTCTT  
 2451 GGAGACCTGG GAGACCTGGT GGACTGAGTA CTGGCAGGCC ACCTGGATCC  
 CCTCTGGACC CTCTGGACCA CCTGACTCAT GACCGTCCGG TGGACCTAGG  
 2501 CTGAGTGGGA GTTTGTGAAC ACCCCCCCCC TGGTGAAGCT GTGGTACCAG  
 GACTCACCTT CAAACACTTG TGGGGGGGGG ACCACTTCGA CACCATGGTC  
 2551 CTGGAGAAGG AGCCCATTGT GGGGGCTGAG ACCTTCTATG TGGCTGGGGC  
 GACCTCTTCC TCGGGTAACA CCCC GACTC TGGGAAGATAC ACCGACCCCG  
 2601 TGCCAACAGG GAGACCAAGC TGGGCAAGGC TGGCTATGTG ACCAACAGGG  
 ACGGTTGTCC CTCTGGTTTCG ACCCGTTCCG ACCGATACAC TGGTTGTCCC  
 2651 GCAGGCAGAA GGTGGTGACC CTGACTGACA CCACCAACCA GAAGACTGCC  
 CGTCCGTCTT CCACCACTGG GACTGACTGT GGTGGTTGGT CTTCTGACGG  
 2701 CTCCAGGCCA TCTACCTGGC CCTCCAGGAC TCTGGCCTGG AGGTGAACAT  
 GAGGTCCGGT AGATGGACCG GGAGGTCTCTG AGACCGGACC TCCACTTGTA  
 2751 TGTGACTGCC TCCCAGTATG CCCTGGGCAT CATCCAGGCC CAGCCTGATC  
 AACTGACGG AGGGTCATAC GGGACCCGTA GTAGGTCCGG GTCGGACTAG

Figure 26C

2851 GAGAAGGTGT ACCTGGCCTG GGTGCCTGCC CACAAGGGCA TTGGGGGCAA  
CTCTTCCACA TGGACCGGAC CCACGGACGG GTGTTCCCGT AACCCCCGTT

2901 TGAGCAGGTG GACAAGCTGG TGTCTGCTGG CATCAGGAAG GTGCTGTTCC  
ACTCGTCCAC CTGTTTCGACC ACAGACGACC GTAGTCTTTC CACGACAAGG

2951 TGGATGGCAT TGACAAGGCC CAGGATGAGC ATGAGAAGTA CCACTCCAAC  
ACCTACCGTA ACTGTTCCGG GTCTTACTCG TACTCTTCAT GGTGAGGTTG

3001 TGGAGGGCTA TGGCCTCTGA CTTCAACCTG CCCCCTGTGG TGGCTAAGGA  
ACCTCCCGAT ACCGGAGACT GAAGTTGGAC GGGGGACACC ACCGATTCTT

3051 GATTGTGGCC TCCTGTGACA AGTGCCAGCT GAAGGGGGAG GCCATGCATG  
CTAACACCGG AGGACACTGT TCACGGTCGA CTTCCCCCTC CGGTACGTAC

3101 GGCAGGTGGA CTGCTCCCTT GGCATCTGGC AGCTGGCCTG CACCCACCTG  
CCGTCCACCT GACGAGGGGA CCGTAGACCG TCGACCGGAC GTGGGTGGAC

3151 GAGGGCAAGG TGATCCTGGT GGCTGTGCAT GTGGCCTCCG GCTACATTGA  
CTCCCGTTCC ACTAGGACCA CCGACACGTA CACCGGAGGC CGATGTAATC

3201 GGCTGAGGTG ATCCCTGCTG AGACAGGCCA GGAGACTGCC TACTTCCTGC  
CCGACTCCAC TAGGGACGAC TCTGTCCGGT CCTCTGACGG ATGAAGGACG

3251 TGAAGCTGGC TGGCAGGTGG CCTGTGAAGA CCATCCACAC TGCCAATGGC  
ACTTCGACCG ACCGTCCACC GGACACTTCT GGTAGGTGTG ACGGTTACCG

3301 TCCAACTTCA CTGGGGCCAC AGTGAGGGCT GCCTGCTGGT GGGCTGGCAT  
AGGTTGAAGT GACCCCGGTG TCACTCCCGA CGGACGACCA CCCGACCGTA

3351 CAAGCAGGAG TTTGGCATCC CCTACAACCC CCAGTCCCAG GGGGTGGTGG  
GTTCTCCTTC AAACCGTAGG GGATGTTGGG GGTGAGGGTC CCCCACCACC

3401 CCTCCATGAA CAAGGAGCTG AAGAAGATCA TTGGGCAGGT GAGGGACCAG  
GGAGGTACTT GTTCCTCGAC TTCTTCTAGT AACCCGTCCA CTCCCTGGTC

3451 GCTGAGCACC TGAAGACAGC TGTGCAGATG GCTGTGTTCA TCCACAACCT  
CGACTCGTGG ACTTCTGTCTG ACACGTCTAC CGACACAAGT AGGTGTTGAA

3501 CAAGAGGAAG GGGGGCATCG GGGGCTACTC CGCTGGGGAG AGGATTGTGG  
GTTCTCCTTC CCCCCTAGC CCCCCTAGC GCGACCCCTC TCCTAACACC

3551 ACATCATTCG CACAGACATC CAGACCAAGG AGCTCCAGAA GCAGATCACC  
TGTAAGTAACG GTGTCTGTAG GTCTGGTTCC TCGAGGTCTT CGTCTAGTGG

3601 AAGATCCAGA ACTTCAGGGT GTACTACAGG GACTCCAGGA ACCCCCTGTG  
TTCTAGGTCT TGAAGTCCCA CATGATGTCC CTGAGGTCTT TGGGGGACAC

3651 GAAGGGCCCT GCCAAGCTGC TGTGGAAGGG GGAGGGGGCT GTGGTGATCC  
CTTCCCGGGA CGGTTTCGACG ACACCTTCCC CCTCCCCGA CACCACTAGG

3701 AGGACAACCTC TGACATCAAG GTGGTGCCCA GGAGGAAGGC CAAGATCATC  
TCCTGTTGAG ACTGTAGTTC CACCACGGGT CCTCCTTCCG GTTCTAGTAG

2 June 26 D

3801 GGATGAGGAC TAAAGCCCGG GCAGATCTGC TGTGCCTTCT AGTTGCCAGC  
CCTACTCCTG ATTTCTGGGCC CGTCTAGACG ACACGGAAGA TCAACGGTCG

3851 CATCTGTTGT TTGCCCCCTC CCCGTGCCTT CCTTGACCCT GGAAGGTGCC  
GTAGACAACA AACGGGGAGG GGGCACGGAA GGAAGTGGGA CCTTCCACGG

3901 ACTCCCACTG TCCTTTCCTA ATAAAATGAG GAAATTGCAT CGCATTGTCT  
TGAGGGTGAC AGGAAAGGAT TATTTTACTC CTTTAACGTA GCGTAACAGA

3951 GAGTAGGTGT CATTCTATTC TGGGGGGTGG GGTGGGGCAG GACAGCAAGG  
CTCATCCACA GTAAGATAAG ACCCCCCACC CCACCCCGTC CTGTCGTTCC

4001 GGGAGGATTG GGAAGACAAT AGCAGGCATG CTGGGGATGC GGTGGGCTCT  
CCCTCCTAAC CCTTCTGTTA TCGTCCGTAC GACCCCTACG CCACCCGAGA

4051 ATGGCCGATC GGC CGCCCGT ACTGAAATGT GTGGGCGTGG CTTAAGGGTG  
TACCGGCTAG CCGCGCGGCA TGACTTTACA CACCCGCACC GAATTCCAC

4101 GGAAAGAATA TATAAGGTGG GGGTCTTATG TAGTTTTGTA TCTGTTTTGC  
CCTTCTTAT ATATTCCACC CCCAGAATAC ATCAAAACAT AGACAAAACG

4151 AGCAGCCGCC GCCGCCATGA GCACCAACTC GTTTGATGGA AGCATTGTGA  
TCGTCGGCGG CGGCGGTACT CGTGGTTGAG CAAACTACCT TCGTAACACT

4201 GCTCATATTT GACAACGCGC ATGCCCCCAT GGGCCGGGGT GCGTCAGAAT  
CGAGTATAAA CTGTTGCGCG TACGGGGGTA CCCGGCCCCA CGCAGTCTTA

4251 GTGATGGGCT CCAGCATTGA TGGTCGCCCC GTCCTGCCCC CAAACTCTAC  
CACTACCCGA GGTCGTAACCT ACCAGCGGGG CAGGACGGGC GTTTGAGATG

4301 TACCTTGACC TACGAGACCG TGTCTGGAAC GCCGTTGGAG ACTGCAGCCT  
ATGGAACCTG ATGCTCTGGC ACAGACCTTG CGGCAACCTC TGACGTCGGA

4351 CCGCCGCCGC TTCAGCCGCT GCAGCCACCG CCCGCGGGAT TGTGACTGAC  
GGCGCGGCGG AAGTCGGCGA CGTCGGTGGC GGGCGCCCTA ACACTGACTG

4401 TTTGCTTTCC TGAGCCCGCT TGCAAACAGT GCAGCTTCCC GTTCATCCGC  
AAACGAAAGG ACTCGGGCGA ACGTTTGTCA CGTCGAAGGG CAAGTAGGCG

4451 CCGCGATGAC AAGTTGACGG CTCTTTTGGC ACAATTGGAT TCTTTGACCC  
GGCGCTACTG TTCAACTGCC GAGAAAACCG TGTTAACCTA AGAAACTGGG

4501 GGGAACTTAA TGTCGTTTCT CAGCAGCTGT TGGATCTGCG CCAGCAGGTT  
CCCTTGAATT ACAGCAAAGA GTCGTCGACA ACCTAGACGC GGTCGTCCAA

4551 TCTGCCCTGA AGGCTTCCTC CCCTCCCAAT GCGGTTTAAA ACATAAATAA  
AGACGGGACT TCCGAAGGAG GGGAGGGTTA CGCCAAATTT TGTATTTATT

4601 AAAACCAGAC TCTGTTTGA TTTGGATCAA GCAAGTGTCT TGCTGTCTTT  
TTTTGGTCTG AGACAAACCT AAACCTAGTT CGTTCACAGA ACGACAGAAA

4651 ATTTAGGGGT TTTGCGCGCG CGGTAGGCCC GGGACCAGCG GTCTCGGTGC  
TAAATCCCCA AAACGCGCGC GCCATCCGGG CCCTGGTCGC CAGAGCCAGC

Figure 26E

4751 GTTCAGATAC ATGGGCATAA GCCCGTCTCT GGGGTGGAGG TAGCACCCT  
CAAGTCTATG TACCCGTATT CGGGCAGAGA CCCACCTCC ATCGTGGTGA

4801 GCAGAGCTTC ATGCTGCGGG GTGGTGTGT AGATGATCCA GTCGTAGCAG  
CGTCTCGAAG TACGACGCCC CACCACAACA TCTACTAGGT CAGCATCGTC

4851 GAGCGCTGGG CGTGGTGCCT AAAAATGTCT TTCAGTAGCA AGCTGATTGC  
CTCGCGACCC GCACCACGGA TTTTACAGA AAGTCATCGT TCGACTAACG

4901 CAGGGGCAGG CCCTTGGTGT AAGTGTTTAC AAAGCGGTGA AGCTGGGATG  
GTCCCCGTCC GGAACCACA TTCACAAATG TTTCGCCAAT TCGACCTAC

4951 GGTGCATACG TGGGGATATG AGATGCATCT TGGACTGTAT TTTTAGGTTG  
CCACGTATGC ACCCCTATAC TCTACGTAGA ACCTGACATA AAAATCCAAC

5001 GCTATGTTCC CAGCCATATC CCTCCGGGGA TTCATGTTGT GCAGAACCAC  
CGATACAAGG GTCGGTATAG GGAGGCCCCCT AAGTACAACA CGTCTTGGTG

5051 CAGCACAGTG TATCCGGTGC ACTTGGGAAA TTTGTCATGT AGCTTAGAAG  
GTCGTGTCAC ATAGGCCACG TGAACCCCTT AAACAGTACA TCGAATCTTC

5101 GAAATGCGTG GAAGAAGTTG GAGACGCCCT TGTGACCTCC AAGATTTTCC  
CTTTACGCAC CTTCTTGAAC CTCTGCGGGA AACTGGAGG TTCTAAAAGG

5151 ATGCATTCTG CCATAATGAT GGCAATGGGC CCACGGGCGG CGGCCTGGGC  
TACGTAAGCA GGTATTACTA CCGTTACCCG GGTGCCCCGC GCCGGACCCG

5201 GAAGATATTT CTGGGATCAC TAACGTCATA GTTGTGTTCC AGGATGAGAT  
CTTCTATAAA GACCCTAGTG ATTGCAGTAT CAACACAAGG TCCTACTCTA

5251 CGTCATAGGC CATTTTACAA AAGCGCGGGC GGAGGGTGCC AGACTGCGGT  
GCAGTATCCG GTAAAAATGT TTCGCGCCCC CCTCCCACGG TCTGACGCCA

5301 ATAATGGTTC CATCCGGCCC AGGGGCGTAG TTACCCTCAC AGATTTGCAT  
TATTACCAAG GTAGGCCGGG TCCCCGCATC AATGGGAGTG TCTAAACGTA

5351 TTCCCACGCT TTGAGTTCAG ATGGGGGGAT CATGTCTACC TCGGGGGCGA  
AAGGGTGCGA AACTCAAGTC TACCCCCCTA GTACAGATGG ACGCCCCGCT

5401 TGAAGAAAAC GGTTCGCGG GTAGGGGAGA TCAGCTGGGA AGAAAGCAGG  
ACTTCTTTTG CCAAAGGCCC CATCCCTCT AGTCGACCCT TCTTTCGTCC

5451 TTCCTGAGCA GCTGCGACTT ACCGCAGCCG GTGGGCCCCG AAATCACACC  
AAGGACTCGT CGACGCTGAA TGGCGTCGGC CACCCGGGCA TTTAGTGTGG

5501 TATTACCGGC TGCAACTGGT AGTTAAGAGA GCTGCAGCTG CCGTCATCCC  
ATAATGGCCG ACGTTGACCA TCAATTCTCT CGACGTCGAC GGCAGTAGGG

5551 TGAGCAGGGG GGCCACTTCG TTAAGCATGT CCCTGACTCG CATGTTTTCC  
ACTCGTCCCC CCGGTGAAGC AATTCGTACA GGGACTGAGC GTACAAAAGG

5601 CTGACCAAAT CCGCCAGAAG GCGCTCGCCG CCCAGCGATA GCAGTTCTTG  
GACTGGTTTA GGCGGTCTTC CGCGAGCGGC GGGTCGCTAT CGTCAAGAAC

Figure 26 F

5701 TTTTGAGCGT TTGACCAAGC AGTTCCAGGC GGTCCCACAG CTCGGTCACC  
 AAAACTCGCA AACTGGTTCG TCAAGGTCCG CCAGGGTGTC GAGCCAGTGG  
 5751 TGCTCTACGG CATCTCGATC CAGCATATCT CCTCGTTTCG CGGGTTGGGG  
 ACGAGATGCC GTAGAGCTAG GTCGTATAGA GGAGCAAAGC GCCCAACCCC  
 5801 CGGCTTTCGC TGTACGGCAG TAGTCGGTGC TCGTCCAGAC GGGCCAGGGT  
 GCCGAAAGCG ACATGCCGTC ATCAGCCACG AGCAGGTCTG CCCGGTCCCA  
 5851 CATGTCTTTC CACGGGCGCA GGGTCCTCGT CAGCGTAGTC TGGGTCACGG  
 GTACAGAAAG GTGCCCGCGT CCCAGGAGCA GTCGCATCAG ACCCAGTGCC  
 5901 TGAAGGGGTG CGCTCCGGGC TGCGCGCTGG CCAGGGTGCG CTTGAGGCTG  
 ACTTCCCCAC GCGAGGCCCG ACGCGCGACC GGTCCCACGC GAACTCCGAC  
 5951 GTCCTGCTGG TGCTGAAGCG CTGCCGGTCT TCGCCCTGCG CGTCGGCCAG  
 CAGGACGACC ACGACTTCGC GACGGCCAGA AGCGGGACGC GCAGCCGGTC  
 6001 GTAGCATTTG ACCATGGTGT CATAGTCCAG CCCCTCCGCG GCGTGGCCCT  
 CATCGTAAAC TGGTACCACA GTATCAGGTC GGGGAGGCGC CGCACCGGGA  
 6051 TGGCGCGCAG CTTGCCCTTG GAGGAGGCGC CGCACGAGGG GCAGTGCAGA  
 ACCGCGCGTC GAACGGGAAC CTCCTCCGCG GCGTGCTCCC CGTCACGTCT  
 6101 CTTTTGAGGG CGTAGAGCTT GGGCGCGAGA AATACCGATT CCGGGGAGTA  
 GAAACTCCC GCATCTCGAA CCCGCGCTCT TTATGGCTAA GGCCCCTCAT  
 6151 GGCATCCGCG CCGCAGGCCC CGCAGACGGT CTCGCATTCC ACGAGCCAGG  
 CCGTAGGCGC GCGTCCGGG GCGTCTGCCA GAGCGTAAGG TGCTCGGTCC  
 6201 TGAGCTCTGG CCGTTCGGGG TCAAAAACCA GGTTCCTCCC ATGCTTTTTG  
 ACTCGAGACC GGCAAGCCCC AGTTTTTGGT CCAAAGGGGG TACGAAAAAC  
 6251 ATGCGTTTCT TACCTCTGGT TTCCATGAGC CGGTGTCCAC GCTCGGTGAC  
 TACGCAAGA ATGGAGACCA AAGGTACTCG GCCACAGGTG CGAGCCACTG  
 6301 GAAAAGGCTG TCCGTGTCCC CGTATACAGA CTTGAGAGGC CTGTCTCGA  
 CTTTTCCGAC AGGCACAGGG GCATATGTCT GAACTCTCCG GACAGGAGCT  
 6351 GCGGTGTTC GCGGTCTTC TCGTATAGAA ACTCGGACCA CTCTGAGACA  
 CGCCACAAGG CGCCAGGAGG AGCATATCTT TGAGCCTGGT GAGACTCTGT  
 6401 AAGGCTCGCG TCCAGGCCAG CACGAAGGAG GCTAAGTGGG AGGGGTAGCG  
 TTCCGAGCGC AGGTCCGGTC GTGCTTCCTC CGATTCAACC TCCCCATCGC  
 6451 GTCGTTGTCC ACTAGGGGGT CCACTCGCTC CAGGGTGTGA AGACACATGT  
 CAGCAACAGG TGATCCCCCA GGTGAGCGAG GTCCCACT TCTGTGTACA  
 6501 CGCCCTCTTC GGCATCAAGG AAGGTGATTG GTTTGTAGGT GTAGGCCACG  
 CGGGGAGAAG CCGTAGTTCC TTCCACTAAC CAAACATCCA CATCCGGTGC  
 6551 TGACCGGGTG TTCCTGAAGG GGGGCTATAA AAGGGGGTGG GGGCGCGTTC  
 ACTGGCCCCAC AAGGACTTCC CCCCAGATATT TTCCCCCACC CCCGCGCAAG

Figure 266

6651 AGTACTCCCT CTGAAAAGCG GGCATGACTT CTGCGCTAAG ATTGTCACTT  
 TCATGAGGGA GACTTTTCGC CCGTACTGAA GACGCGATTG TAACAGTCAA  
 6701 TCCAAAAACG AGGAGGATTT GATATTCACC TGGCECGCGG TGATGCCTTT  
 AGGTTTTTGC TCCTCCTAAA CTATAAGTGG ACCGGGCGCC ACTACGAAAA  
 6751 GAGGGTGGCC GCATCCATCT GGTGAGAAAA GACAATCTTT TTGTTGTCAA  
 CTCCCACCGG CGTAGGTAGA CCAGTCTTTT CTGTTAGAAA AACACAGTT  
 6801 GCTTGGTGGC AAACGACCCG TAGAGGGCGT TGGACAGCAA CTTGGCGATG  
 CGAACCACCG TTTGCTGGGC ATCTCCCGCA ACCTGTCGTT GAACCGCTAC  
 6851 GAGCGCAGGG TTTGGTTTTT GTCGCGATCG GCGCGCTCCT TGGCCGCGAT  
 CTCGCGTCCC AAACCAAAAA CAGCGCTAGC CGCGCGAGGA ACCGGCGCTA  
 6901 GTTTAGCTGC ACGTATTGCG GCGCAACGCA CCGCCATTCT GGAAAGACGG  
 CAAATCGACG TGCATAAGCG CGCGTTGCGT GCGGTAAGC CCTTCTGCGC  
 6951 TGGTGCCTGC GTCGGGCACC AGGTGCACGC GCCAACCGCG GTTGTGCAGG  
 ACCACGCGAG CAGCCCGTGG TCCACGTGCG CGGTTGGCGC CAACACGTCC  
 7001 GTGACAAGGT CAACGCTGGT GGCTACCTCT CCGCGTAGGC GCTCGTTGGT  
 CACTGTTCCA GTTGCAGCCA CCGATGGAGA GCGCGATCCG CGAGCAACCA  
 7051 CCAGCAGAGG CGGCCGCCCT TCGCGAGCA GAATGGCGGT AGGGGGTCTA  
 GGTGCTCTCC GCCGGCGGGA ACGCGCTCGT CTTACCGCCA TCCCCAGAT  
 7101 GCTGCGTCTC GTCCGGGGGG TCTGCGTCCA CGGTAAAGAC CCCGGGCAGC  
 CGACGCAGAG CAGGCCCCCC AGACGCAGGT GCCATTTCTG GGGCCCGTCG  
 7151 AGGCGCGCGT CGAAGTAGTC TATCTTGCAT CTTGCAAGT CTAGCGCCTG  
 TCCGCGCGCA GCTTCATCAG ATAGAACGTA GGAACGTTCA GATCGCGGAC  
 7201 CTGCCATGCG CGGGCGGCAA GCGCGCGCTC GTATGGGTTG AGTGGGGGAC  
 GACGGTACGC GCCCGCCGTT CGCGCGCGAG CATACCCAAC TCACCCCTG  
 7251 CCCATGGCAT GGGGTGGGTG AGCGCGGAGG CGTACATGCC GCAAATGTGG  
 GGGTACCGTA CCCACCCAC TCGCGCCTCC GCATGTACGG CGTTTACAGC  
 7301 TAAACGTAGA GGGGCTCTCT GAGTATTCCA AGATATGTAG GGTAGCATCT  
 ATTTGCATCT CCCCAGAGA CTCATAAGGT TCTATACATC CCATCGTAGA  
 7351 TCCACCGCGG ATGCTGGCGC GCACGTAATC GTATAGTTCT TGCGAGGGAG  
 AGGTGGCGCC TACGACCGCG CGTGCATTAG CATATCAAGC ACGTCCCTC  
 7401 CGAGGAGGTC GGGACCGAGG TTGCTACGGG CGGGCTGCTC TGCTCGGAAG  
 GCTCCTCCAG CCCTGGCTCC AACGATGCCC GCCCGACGAG ACGAGCCTTC  
 7451 ACTATCTGCC TGAAGATGGC ATGTGAGTTG GATGATATGG TTGGACGCTG  
 TGATAGACGG ACTTCTACCG TACACTCAAC CTACTATACC AACCTGCGAC  
 7501 GAAGACGTTG AAGCTGGCGT CTGTGAGACC TACCGCGTCA CGCAGGAAGG  
 CTTCTGCAAC TTCGACCGCA GACACTCTGG ATGGCGCAGT GCGTGCTTCC

Figure 26 H

7601 TCTAGGGCGC AGTAGTCCAG GGTTCCTTG ATGATGTCAT ACTTATCCTG  
 AGATCCC GCG TCATCAGGTC CCAAAGGAAC TACTACAGTA TGAATAGGAC  
 7651 TCCCTTTTTT TTCCACAGCT CGCGGTTGAG GACAACTCT TCGCGGTCTT  
 AGGGAAAAA AAGGTGTCGA GCGCCAATC CTGTTTGAGA AGCGCCAGAA  
 7701 TCCAGTACTC TTGGATCGGA AACCCGTCGG CCTCCGAACG GTAAGAGCCT  
 AGGTCATGAG AACCTAGCCT TTGGGCAGCC GGAGGCTTGC CATTCCTCGGA  
 7751 AGCATGTAGA ACTGGTTGAC GGCCTGGTAG GCGCAGCATC CCTTTTCTAC  
 TCGTACATCT TGACCAACTG CCGGACCATC CGCGTCGTAG GGAAAAGATG  
 7801 GGGTAGCGCG TATGCCTGCG CGGCCTTCCG GAGCGAGGTG TGGGTGAGCG  
 CCCATCGCGC ATACGGACGC GCCGGAAGGC CTCGCTCCAC ACCCACTCGC  
 7851 CAAAGGTGTC CCTGACCATG ACTTTGAGGT ACTGGTATTT GAAGTCAGTG  
 GTTTCCACAG GGA CTGGTAC TGAAACTCCA TGACCATAAA CTTCAGTCAC  
 7901 TCGTCGCATC CGCCCTGCTC CCAGAGCAAA AAGTCCGTGC GCTTTTTGGA  
 AGCAGCGTAG GCGGGACGAG GGTCTCGTTT TTCAGGCACG CGAAAAACCT  
 7951 ACGCGGATTT GGCAGGGCGA AGGTGACATC GTTGAAGAGT ATCTTTCCCG  
 TGCGCCTAAA CCGTCCC GCT TCCACTGTAG CAACTTCTCA TAGAAAGGGC  
 8001 CGCGAGGCAT AAAGTTGCGT GTGATGCGGA AGGGTCCCGG CACCTCGGAA  
 GCGCTCCGTA TTTCAACGCA CACTACGCCT TCCCAGGGCC GTGGAGCCTT  
 8051 CGGTGTGTTAA TTACCTGGGC GGCAGGCACG ATCTCGTCAA AGCCGTTGAT  
 GCCAACAAAT AATGGACCCG CCGCTCGTGC TAGAGCAGTT TCGGCAACTA  
 8101 GTTGTGCCCC ACAATGTAAA GTTCCAAGAA GCGCGGGATG CCCTTGATGG  
 CAACACCGGG TGTTACATTT CAAGGTTCCT CCGCCCTTAC GGGA ACTACC  
 8151 AAGGCAATTT TTTAAGTTCC TCGTAGGTGA GCTCTTCAGG GGAGCTGAGC  
 TTCCGTTAAA AAATCAAGG AGCATCCACT CGAGAAGTCC CCTCGACTCG  
 8201 CCGTGCTCTG AAAGGGCCCA GTCTGCAAGA TGAGGGTTGG AAGCGACGAA  
 GGCACGAGAC TTTCCCGGGT CAGACGTTCT ACTCCCAACC TTCGCTGCTT  
 8251 TGAGCTCCAC AGGTACCGG CCATTAGCAT TTGCAGGTGG TCGCGAAAGG  
 ACTCGAGGTG TCCAGTGCCC GGTAATCGTA AACGTCCACC AGCGCTTTCC  
 8301 TCCTAAACTG GCGACCTATG GCCATTTTTT CTGGGGTGAT GCAGTAGAAG  
 AGGATTTGAC CGCTGGATAC CGGTAAAAA GACCCCACTA CGTCATCTTC  
 8351 GTAAGCGGGT CTTGTTCCCA GCGGTCCCAT CCAAGGTTCG CGGCTAGGTC  
 CATTCGCCCC GAACAAGGGT CGCCAGGGTA GGTCCAAGC GCCGATCCAG  
 8401 TCGCGCGGCA GTCAGTAGAG GCTCATCTCC GCCGAACCTC ATGACCAGCA  
 AGCGCGCCGT CAGTGATCTC CGAGTAGAGG CGGCTTGAAG TACTGGTCGT  
 8451 TGAAGGGCAC GAGCTGCTTC CCAAAGGCC CCATCCAAGT ATAGGTCTCT  
 ACTTCCCGTG CTCGACGAAG GGTTCGCGG GGTAGGTTCA TATCCAGAGA

*Figure 26 I*

8551 GAAGAAGTGG ATCTCCCGCC ACCAATTGGA GGAGTGGCTA TTGATGTGGT  
CTTCTTGACC TAGAGGGCGG TGGTTAACCT CCTCACCAGT AACTACACCA

8601 GAAAGTAGAA GTCCCTGCGA CGGGCCGAAC ACTCGTGCTG GCTTTTGTAA  
CTTTCATCTT CAGGGACGCT GCCCGGCTTG TGAGCACGAC CGAAAACATT

8651 AAACGTGCGC AGTACTGGCA GCGGTGCACG GGCTGTACAT CCTGCACGAG  
TTTGCACGCG TCATGACCGT CGCCACGTGC CCGACATGTA GGACGTGCTC

8701 GTTGACCTGA CGACCGCGCA CAAGGAAGCA GAGTGGGAAT TTGAGCCCCT  
CAACTGGACT GCTGGCGCGT GTTCCTTCGT CTCACCCCTA AACTCGGGGA

8751 CGCCTGGCGG GTTTGGCTGG TGGTCTTCTA CTTCGGCTGC TTGTCTTGA  
GCGGACCGCC CAAACCGACC ACCAGAAGAT GAAGCCGACG AACAGGAACT

8801 CCGTCTGGCT GCTCGAGGGG AGTTACGGTG GATCGGACCA CCACGCCGCG  
GGCAGACCGA CGAGCTCCCC TCAATGCCAC CTAGCCTGGT GGTGCGGCGC

8851 CGAGCCCCAA GTCCAGATGT CCGCGCGCGG CGGTGCGAGC TTGATGACAA  
GCTCGGGTTT CAGGTCTACA GCGCGCGCGC GCCAGCCTCG AACTACTGTT

8901 CATCGCGCAG ATGGGAGCTG TCCATGGTCT GGAGCTCCCG CGGCGTCAGG  
GTAGCGCGTC TACCCTCGAC AGGTACCAGA CCTCGAGGGC GCCGCAGTCC

8951 TCAGGCGGGA GCTCCTGCAG GTTTACCTCG CATAGACGGG TCAGGGCGCG  
AGTCCGCCCT CGAGGACGTC CAAATGGAGC GTATCTGCCC AGTCCGCGC

9001 GGCTAGATCC AGGTGATACC TAATTTCCAG GGGCTGGTTG GTGGCGGCGT  
CCGATCTAGG TCCACTATGG ATTAAAGGTC CCCGACCAAC CACCGCCGCA

9051 CGATGGCTTG CAAGAGGCCG CATCCCCGCG GCGCGACTAC GGTACCGCGC  
GCTACCGAAC GTTCTCCGGC GTAGGGGCGC CGCGCTGATG CCATGGCGCG

9101 GGCGGGCGGT GGGCCGCGGG GGTGTCCTTG GATGATGCAT CTAAAAGCGG  
CCGCCGCCCA CCCGGCGCCC CCACAGGAAC CTACTACGTA GATTTTCGCC

9151 TGACGCGGGC GAGCCCCCGG AGGTAGGGGG GGCTCCGGAC CCGCCGGGAG  
ACTGCGCCCC CTCGGGGGCC TCCATCCCCC CCGAGGCCTG GCGGGCCCTC

9201 AGGGGGCAGG GGCACGTGCG CGCCGCGCGC GGGCAGGAGC TGGTGCTGCG  
TCCCCCGTCC CCGTGCAGCC GCGGCGCGCG CCCGTCCTCG ACCACGACGC

9251 CGCGTAGGTT GCTGGCGAAC GCGACGACGC GCGGTTGAT CTCCTGAATC  
GCGCATCCAA CGACCGCTTG CGCTGCTGCG CCGCCAATA GAGGACTTAG

9301 TGGCGCCTCT GCGTGAAGAC GACGGGCCCC GTGAGCTTGA ACCTGAAAGA  
ACCGCGGAGA CGCACTTCTG CTGCCCCGGC CACTCGAACT TGGACTTTCT

9351 GAGTTCGACA GAATCAATTT CGGTGTGCTT GACGGCGGCC TGGCGCAAAA  
CTCAAGCTGT CTTAGTTAAA GCCACAGCAA CTGCCGCCGG ACCGCGTTTT

9401 TCTCCTGCAC GTCTCCTGAG TTGTCTTGAT AGGCGATCTC GGCCATGAAC  
AGAGGACGTG CAGAGGACTC AACAGAACTA TCCGCTAGAG CCGGTACTTG

Figure 26 J



9501 GGGGGCGAGG TCGTTGGAAA TGCGGGCCAT GAGCTGCGAG AAGGCGTTGA  
CCGCCGCTCC AGCAACCTTT ACGCCCGGTA CTCGACGCTC TTCCGCAACT

9551 GGCCTCCCTC GTTCCAGACG CGGCTGTAGA CCACGCCCCC TTCGGCATCG  
CCGGAGGGAG CAAGGTCTGC GCCGACATCT GGTGCGGGGG AAGCCGTAGC

9601 CGGGCGCGCA TGACCACCTG CGCGAGATTG AGCTCCACGT GCCGGGCGAA  
GCCCCGCGCT ACTGGTGGAC GCGCTCTAAC TCGAGGTGCA CGGCCCGCTT

9651 GACGGCGTAG TTTTCGAGGC GCTGAAAGAG GTAGTTGAGG GTGGTGGCGG  
CTGCCGCATC AAAGCGTCCG CGACTTTCTC CATCAACTCC CACCACCGCC

9701 TGTGTTCTGC CACGAAGAAG TACATAACCC AGCGTCGCAA CGTGGATTCTG  
ACACAAGACG GTGCTTCTTC ATGTATTGGG TCGCAGCGTT GCACCTAAGC

9751 TTGATATCCC CCAAGGCCCTC AAGGCGCTCC ATGGCCTCGT AGAAGTCCAC  
AACTATAGGG GGTTCGGAG TTCCGCGAGG TACCGGAGCA TCTTCAGGTG

9801 GGGGAAGTTG AAAAAGTGGG AGTTGCGCGC CGACACGGTT AACTCCTCCT  
CCGCTTCAAC TTTTGTACCC TCAACGCGCG GCTGTGCCAA TTGAGGAGGA

9851 CCAGAAGACG GATGAGCTCG GCGACAGTGT CGCGCACCTC GCGCTCAAAG  
GGTCTTCTGC CTACTCGAGC CGCTGTCACA GCGCGTGGAG CGCGAGTTTC

9901 GCTACAGGGG CCTCTTCTTC TTCTTCAATC TCCTCTTCCA TAAGGGCCTC  
CGATGTCCCC GGAGAAGAAG AAGAAGTTAG AGGAGAAGGT ATTCCCGAG

9951 CCCTTCTTCT TCTTCTGGCG GCGGTGGGGG AGGGGGGACA CGGCGGCGAC  
GGGAAGAAGA AGAAGACCGC CGCCACCCCC TCCCCCTGT GCCGCCGCTG

10001 GACGGCGCAC CGGGAGGCGG TCGACAAAGC GCTCGATCAT CTCCCCGCGG  
CTGCCGCGTG GCCCTCCGCC AGCTGTTTCG CGAGCTAGTA GAGGGGCGCC

10051 CGACGGCGCA TGGTCTCGGT GACGGCGCGG CCGTTCTCGC GGGGGCGCAG  
GCTGCCGCGT ACCAGAGCCA CTGCCGCGCC GGCAAGAGCG CCCCCGCGTC

10101 TTGGAAGACG CCGCCCGTCA TGTCCCGGTT ATGGGTTGGC GGGGGGCTGC  
AACCTTCTGC GCGGGGCAGT ACAGGGCCAA TACCCAACCG CCCCCGACG

10151 CATGCGGCAG GGATACGGCG CTAACGATGC ATCTCAACAA TTGTTGTGTA  
GTACGCCGTC CCTATGCCGC GATTGCTACG TAGAGTTGTT AACAAACAT

10201 GGTACTCCGC CGCCGAGGGA CCTGAGCGAG TCCGCATCGA CCGGATCGGA  
CCATGAGGCG GCGGCTCCCT GGACTCGCTC AGGCGTAGCT GGCCTAGCCT

10251 AAACCTCTCG AGAAAGGCGT CTAACGATC ACAGTCGCAA GGTAGGCTGA  
TTTGGAGAGC TCTTTCCGCA GATTGGTCAG TGTCAGCGTT CCATCCGACT

10301 GCACCGTGGC GGGCGGCAGC GGGCGGCGGT CGGGGTTGTT TCTGGCGGAG  
CGTGGCACCG CCCGCCGTCG CCCGCCGCCA GCCCAACAA AGACCGCCTC

10351 GTGCTGCTGA TGATGTAATT AAAGTAGGCG GTCTTGAGAC GGCGGATGGT  
CACGACGACT ACTACATTAA TTTCATCCGC CAGAACTCTG CCGCCTACCA

Figure 26 K

10451 CGGCCATGCC CCAGGCTTCG TTTTGACATC GGCGCAGGTC TTTGTTAGTAG  
 GCCGGTACGG GGTCCGAAGC AAAACTGTAG CCGCGTCCAG AAACATCATC  
 10501 TCTTGCATGA GCCTTTCTAC CGGCACTTCT TCTTCTCCTT CCTCTTGTCC  
 AGAACGTACT CGGAAAGATG GCCGTGAAGA AGAAGAGGAA GGAGAACAGG  
 10551 TGCATCTCTT GCATCTATCG CTGCGGCGGC GGCGGAGTTT GGCCGTAGGT  
 ACGTAGAGAA CGTAGATAGC GACGCCGCCG CCGCCTCAAA CCGGCATCCA  
 10601 GGCGCCCTCT TCCTCCCATG CGTGTGACCC CGAAGCCCTT CATCGGCTGA  
 CCGCGGGAGA AGGAGGGTAC GCACACTGGG GCTTCGGGGA GTAGCCGACT  
 10651 AGCAGGGGCTA GGTCGGCGAC AACGCGCTCG GCTAATATGG CCTGCTGCAC  
 TCGTCCCGAT CCAGCCGCTG TTGCGCGAGC CGATTATACC GGACGACGTG  
 10701 CTGCGTGAGG GTAGACTGGA AGTCATCCAT GTCCACAAAG CCGTGGTATG  
 GACGCACTCC CATCTGACCT TCAGTAGGTA CAGGTGTTTC GCCACCATA  
 10751 CGCCCGTGTT GATGGTGTA GTGCAGTTGG CCATAACGGA CCAGTTAACG  
 GCGGGCACAA CTACCACATT CACGTCAACC GGTATTGCCT GTCAATTGC  
 10801 GTCTGGTGAC CCGGCTGCGA GAGCTCGGTG TACCTGAGAC GCGAGTAAGC  
 CAGACCACTG GGCCGACGCT CTCGAGCCAC ATGGACTCTG CGCTCATTCG  
 10851 CCTCGAGTCA AATACGTAGT CGTTGCAAGT CCGCACCAGG TACTGGTATC  
 GGAGCTCAGT TTATGCATCA GCAACGTTCA GGCGTGGTCC ATGACCATAG  
 10901 CCACCAAAAA GTGCGGCGGC GGCTGGCGGT AGAGGGGCCA GCGTAGGGTG  
 GGTGGTTTTT CACGCCGCCG CCGACCGCCA TCTCCCCGGT CGCATCCAC  
 10951 GCCGGGGCTC CGGGGGCGAG ATCTTCCAAC ATAAGGCGAT GATATCCGTA  
 CGGCCCGGAG GCGCCCGCTC TAGAAGGTTG TATTCGCTA CTATAGGCAT  
 11001 GATGTACCTG GACATCCAGG TGATGCCGGC GGCGGTGGTG GAGGCGCGCG  
 CTACATGGAC CTGTAGGTCC ACTACGGCCG CCGCCACCAC CTCCGCGCGC  
 11051 GAAAGTCGCG GACGCGGTTT CAGATGTTGC GCAGCGGCAA AAAGTGCTCC  
 CTTTCAGCGC CTGCGCCAAG GTCTACAACG CGTCGCCGTT TTTCACGAGG  
 11101 ATGGTCGGGA CGCTCTGGCC GGTCAGGCGC GCGCAATCGT TGACGCTCTA  
 TACCAGCCCT GCGAGACCGG CCAGTCCGCG CGCGTTAGCA ACTGCGAGAT  
 11151 GACCGTGCAA AAGGAGAGCC TGTAAGCGGG CACTCTTCCG TGGTCTGGTG  
 CTGGCACGTT TTCTCTCGG ACATTCGCCC GTGAGAAGGC ACCAGACCAC  
 11201 GATAAATTCG CAAGGGTATC ATGGCGGACG ACCGGGGTTC GAGCCCCGTA  
 CTATTTAAGC GTTCCCATAG TACCGCCTGC TGGCCCCAAG CTCGGGGCAT  
 11251 TCCGGCCGTC CGCCGTGATC CATGCGGTTA CCGCCCGCGT GTCGAACCCA  
 AGGCCGGCAG GCGGCACTAG GTACGCCAAT GCGGGGCGCA CAGCTTGGGT  
 11301 GGTGTGCGAC GTCAGACAAC GGGGGAGTGC TCCTTTTGGC TTCCTTCCAG  
 CCACACGCTG CAGTCTGTTG CCCCCACG AGGAAAACCG AAGGAAGGTG

Figure 26L

11401 AAGCGGTTAG GCTGGAAAGC GAAAGCATTAG AGTGGCTCGC TCCCTGTAGC  
TTCGCCAATC CGACCTTTTCG CTTTCGTAAT TCACCGAGCG AGGGACATCG

11451 CGGAGGGTTA TTTTCCAAGG GTTGAGTCGC GGGACCCCCG GTTCGAGTCT  
GCCTCCCAAT AAAAGGTTCC CAACTCAGCG CCCTGGGGGC CAAGCTCAGA

11501 CGGACCGGCC GGA CTGCGGC GAACGGGGGT TTGCCTCCCC GTCATGCAAG  
GCCTGGCCGG CCTGACGCCG CTTGCCCCCA AACGGAGGGG CAGTACGTTT

11551 ACCCCGCTTG CAAATTCCTC CGGAAACAGG GACGAGCCCC TTTTTTGCTT  
TGGGGCGAAC GTTTAAGGAG GCCTTTGTCC CTGCTCGGGG AAAAAACGAA

11601 TTCCAGATG CATCCGGTGC TCGGCAGAT GCGCCCCCT CCTCAGCAGC  
AAGGGTCTAC GTAGGCCACG ACGCCGTCTA CCGGGGGGA GGAGTCGTCG

11651 GGCAAGAGCA AGAGCAGCGG CAGACATGCA GGGCACCTC CCCTCCTCCT  
CCGTTCTCGT TCTCGTCGCC GTCTGTACGT CCCGTGGGAG GGGAGGAGGA

11701 ACCGCGTCAG GAGGGGCGAC ATCCGCGGTT GACGCGGCAG CAGATGGTGA  
TGGCGCAGTC CTCCCCGCTG TAGGCGCCAA CTGCGCCGTC GTCTACCACT

11751 TTACGAACCC CCGCGGCGCC GGGCCCGGCA CTACCTGGAC TTGGAGGAGG  
AATGCTTGGG GGCGCCGCGG CCCGGGCCGT GATGGACCTG AACCTCCTCC

11801 GCGAGGGCCT GCGCGGGCTA GGAGCGCCCT CTCCTGAGCG GCACCCAAGG  
CGCTCCCGGA CCGCGCCGAT CCTCGCGGGA GAGGACTCGC CGTGGGTTC

11851 GTGCAGCTGA AGCGTGATAC GCGTGAGGCG TACGTGCCGC GGCAGAACCT  
CACGTGCACT TCGCACTATG CGCACTCCGC ATGCACGGCG CCGTCTTGGA

11901 GTTTCGCGAC CGCGAGGGAG AGGAGCCCGA GGAGATGCGG GATCGAAAGT  
CAAAGCGCTG GCGCTCCCTC TCCTCGGGCT CCTCTACGCC CTAGCTTTCA

11951 TCCACGCAGG GCGCGAGCTG CGGCATGGCC TGAATCGCGA GCGGTTGCTG  
AGGTGCGTCC CGCGCTCGAC GCCGTACCG ACTTAGCGCT CGCCAACGAC

12001 CGCGAGGAGG ACTTTGAGCC CGACGCGCGA ACCGGGATTA GTCCCGCGCG  
GCGCTCCTCC TGAAACTCGG GCTGCGCGCT TGGCCCTAAT CAGGGCGCGC

12051 CGCACACGTG GCGGCCGCCG ACCTGGTAAC CGCATACGAG CAGACGGTGA  
GCGTGTGCAC CGCCGGCGGC TGGACCATG GCGTATGCTC GTCTGCCACT

12101 ACCAGGAGAT TAACTTTCAA AAAAGCTTTA ACAACCACGT GCGTACGCTT  
TGGTCTCTA ATTGAAAGTT TTTTCGAAAT TGTTGGTGCA CGCATGCGAA

12151 GTGGCGCGCG AGGAGGTGGC TATAGGACTG ATGCATCTGT GGGACTTTGT  
CACC GC GCGC TCCTCCACCG ATATCCTGAC TACGTAGACA CCCTGAAACA

12201 AAGCGCGCTG GAGCAAAACC CAAATAGCAA GCCGCTCATG GCGCAGCTGT  
TTCGCGCGAC CTCGTTTTGG GTTTATCGTT CGGCGAGTAC CCGGTCGACA

12251 TCCTTATAGT GCAGCACAGC AGGGACAACG AGGCATTAG GGATGCGCTG  
AGGAATATCA CGTCGTGTCG TCCCTGTTGC TCCGTAAGTC CCTACGCGAC

Figure 26 M

12351 CCTGCAGAGC ATAGTGGTGC AGGAGCGCAG CTTGAGCCTG GCTGACAAGG  
GGACGTCTCG TATCACCACG TCCTCGCGTC GAACTCGGAC CGACTGTTCC

12401 TGGCCGCCAT CAACTATTCC ATGCTTAGCC TGGGCAAGTT TTACGCCCCG  
ACCGCGGTA GTTGATAAGG TACGAATCGG ACCCGTTCAA AATGCGGGCG

12451 AAGATATACC ATACCCCTTA CGTTCCCATA GACAAGGAGG TAAAGATCGA  
TTCTATATGG TATGGGGAAT GCAAGGTAT CTGTTCTCC ATTTCTAGCT

12501 GGGGTTCTAC ATGCGCATGG CGCTGAAGGT GCTTACCTTG AGCGACGACC  
CCCCAAGATG TACGCGTACC GCGACTTCCA CGAATGGAAC TCGCTGCTGG

12551 TGGGCGTTTA TCGCAACGAG CGCATCCACA AGGCCGTGAG CGTGAGCCGG  
ACCGCGAAAT AGCGTTGCTC GCGTAGGTGT TCCGGCACTC GCACTCGGCC

12601 CGGCGCGAGC TCAGCGACCG CGAGCTGATG CACAGCCTGC AAAGGGCCCT  
GCCGCGCTCG AGTCGCTGGC GCTCGACTAC GTGTCGGACG TTCCCGGGA

12651 GGCTGGCACG GGCAGCGGCG ATAGAGAGGC CGAGTCCTAC TTTGACGCGG  
CCGACCGTGC CCGTCGCCGC TATCTCTCCG GCTCAGGATG AAAC TGCGCC

12701 GCGCTGACCT GCGCTGGGCC CCAAGCCGAC GCGCCCTGGA GGCAGCTGGG  
CGCGACTGGA CGCGACCCGG GGTTCGGCTG CGCGGGACCT CCGTCGACCC

12751 GCCGGACCTG GGCTGGCGGT GGCACCCGCG CGCGCTGGCA ACGTCGGCGG  
CGGCCTGGAC CCGACCGCCA CCGTGGGCGC GCGCGACCGT TGCAGCCGCC

12801 CGTGGAGGAA TATGACGAGG ACGATGAGTA CGAGCCAGAG GACGGCGAGT  
GCACCTCCTT ATACTGCTCC TGCTACTCAT GCTCGGTCTC CTGCCGCTCA

12851 ACTAAGCGGT GATGTTTCTG ATCAGATGAT GCAAGACGCA ACGGACCCGG  
TGATTGCGCA CTACAAAGAC TAGTCTACTA CGTTCTGCGT TGCTTGGGCC

12901 CGGTGCGGGC GGCCTGCGAG AGCCAGCCGT CCGGCCTTAA CTCCACGGAG  
GCCACGCCCC CCGCGACGTC TCGGTCGGCA GGCCGGAATT GAGGTGCCTG

12951 GACTGGCGCC AGGTCATGGA CCGCATCATG TCGCTGACTG CGCGCAATCC  
CTGACCGCGG TCCAGTACCT GCGGTAGTAC AGCGACTGAC GCGCGTTAGG

13001 TGACGCGTTC CGGCAGCAGC CGCAGGCCAA CCGGCTCTCC GCAATTCTGG  
ACTGCGCAAG GCCGTCGTCG GCGTCCGGTT GGCCGAGAGG CGTTAAGACC

13051 AAGCGGTGGT CCCGGCGCGC GCAAACCCCA CGCACGAGAA GGTGCTGGCG  
TTCGCCACCA GGGCCGCGCG CGTTTGGGGT GCGTGCTCTT CCACGACCGC

13101 ATCGTAAACG CGCTGGCCGA AAACAGGGCC ATCCGGCCCCG ACGAGGCCGG  
TAGCATTTGC GCGACCGGCT TTTGTCCCGG TAGGCCGGGC TGCTCCGGCC

13151 CCTGGTCTAC GACGCGCTGC TTCAGCGCGT GGCTCGTTAC AACAGCGGCA  
GGACCAGATG CTGCGCGACG AAGTCGCGCA CCGAGCAATG TTGTGCGCGT

13201 ACGTGACAGC CAACCTGGAC CGGCTGGTGG GGGATGTGCG CGAGGCCGTG  
TGACGCTCTG GTTGGACCTG GCCGACCACC CCCTACACGC GCTCCGGCAC

Figure 26 N

13301 ACTAAACGCC TTCCTGAGTA CACAGCCCGC CAACGTGCCG CGGGGACAGG  
TGATTTGCGG AAGGACTCAT GTGTCGGGCG GTTGACACGGC GCCCCTGTCC

13351 AGGACTACAC CAACTTTGTG AGCGCACTGC GGCTAATGGT GACTGAGACA  
TCCTGATGTG GTTGAAACAC TCGCGTGACG CCGATTACCA CTGACTCTGT

13401 CCGCAAAGTG AGGTGTACCA GTCTGGGCCA GACTATTTTT TCCAGACCAG  
GGCGTTTCAC TCCACATGGT CAGACCCGGT CTGATAAAAA AGGTCTGGTC

13451 TAGACAAGGC CTGCAGACCG TAAACCTGAG CCAGGCTTTC AAAAAGTTGC  
ATCTGTTCCG GACGTCTGGC ATTTGGACTC GGTCCGAAAG TTTTGAACG

13501 AGGGGCTGTG GGGGGTGCGG GCTCCCACAG GCGACCGCGC GACCGTGTCT  
TCCCCGACAC CCCCCACGCC CGAGGGTGTC CGCTGGCGCG CTGGCACAGA

13551 AGCTTGCTGA CGCCCAACTC GCGCCTGTTG CTGCTGCTAA TAGCGCCCTT  
TCGAACGACT GCGGGTTGAG CGCGGACAAC GACGACGATT ATCGCGGGAA

13601 CACGGACAGT GGCAGCGTGT CCCGGGACAC ATACCTAGGT CACTTGCTGA  
GTGCCTGTCA CCGTCGCACA GGGCCCTGTG TATGGATCCA GTGAACGACT

13651 CACTGTACCG CGAGGCCATA GGTCAGGCGC ATGTGGACGA GCATACTTTC  
GTGACATGGC GCTCCGGTAT CCAGTCCGCG TACACCTGCT CGTATGAAAG

13701 CAGGAGATTA CAAGTGTCAG CCGCGCGCTG GGGCAGGAGG ACACGGGCAG  
GTCCTCTAAT GTTCACAGTC GGC GCGCGAC CCCGTCTCC TGTGCCCCTC

13751 CCTGGAGGCA ACCCTAACT ACCTGCTGAC CAACCGGCGG CAGAAGATCC  
GGACCTCCGT TGGGATTTGA TGGACGACTG GTTGGCCGCC GTCTTCTAGG

13801 CCTCGTTGCA CAGTTTAAAC AGCGAGGAGG AGCGCATTTT GCGCTACGTG  
GGAGCAACGT GTCAAATTTG TCGCTCCTCC TCGCGTAAA CGCGATGCAC

13851 CAGCAGAGCG TGAGCCTTAA CCTGATGCGC GACGGGGTAA CGCCCAGCGT  
GTGCTCTCGC ACTCGGAATT GGA CTGACGCG CTGCCCCATT GCGGGTCGCA

13901 GCGCTGGAC ATGACCGCGC GCAACATGGA ACCGGGCATG TATGCCTCAA  
CCGCGACCTG TACTGGCGCG CGTTGTACCT TGGCCCGTAC ATACGGAGTT

13951 ACCGGCCGTT TATCAACCGC CTAATGGACT ACTTGCTGCG CGCGGCCGCC  
TGGCCGGCAA ATAGTTGGCG GATTACCTGA TGAACGTAGC GCGCCGGCGG

14001 GTGAACCCCG AGTATTTAC CAATGCCATC TTGAACCCCG ACTGGCTACC  
CACTTGGGGC TCATAAAGTG GTTACGGTAG AACTTGGGCG TGACCGATGG

14051 GCCCCCTGGT TTCTACACCG GGGGATTGCA GGTGCCCAG GGTAAACGATG  
CGGGGGACCA AAGATGTGGC CCCCTAAGCT CCACGGGCTC CCATTGCTAC

14101 GATTCTCTG GGACGACATA GACGACAGCG TGTTTTCCCC GCAACCGCAG  
CTAAGGAGAC CCTGCTGTAT CTGCTGTCGC ACAAAGGGG CGTTGGCGTC

14151 ACCCTGCTAG AGTTGCAACA GCGCGAGCAG GCAGAGGCGG CGCTGCGAAA  
TGGGACGATC TCAACGTTGT CCGCTCTGTC CGTCTCCGCC GCGACGCTTT

Figure 260

14251 CGCGGTCAGA TGCTAGTAGC CCATTTCCTAA GCTTGATAGG GTCTCTTACC  
GCGCCAGTCT ACGATCATCG GGTAAAGGTT CGAACTATCC CAGAGAATGG

14301 AGCACTCGCA CCACCCGCCC GCGCCTGCTG GCGGAGGAGG AGTACCTAAA  
TCGTGAGCGT GGTGGGCGGG CCGGACGAC CCGCTCCTCC TCATGGATTT

14351 CAACTCGCTG CTGCAGCCGC AGCGCGAAAA AAACCTGCCT CCGGCATTTT  
GTTGAGCGAC GACGTCGGCG TCGCGCTTTT TTTGGACGGA GGCCGTAAAG

14401 CCAACAACGG GATAGAGAGC CTAGTGGACA AGATGAGTAG ATGGAAGACG  
GGTTGTTGCC CTATCTCTCG GATCACCTGT TCTACTCATC TACCTTCTGC

14451 TACGCGCAGG AGCACAGGGA CGTGCCAGGC CCGCGCCC GC CCACCCGTCG  
ATGCGCGTCC TCGTGTCCCT GCACGGTCCG GCGCGGGCG GGTGGGCAGC

14501 TCAAAGGCAC GACCGTCAGC GGGGTCTGGT GTGGGAGGAC GATGACTCGG  
AGTTTCCGTG CTGGCAGTCG CCCAGACCA CACCTCCTG CTACTGAGCC

14551 CAGACGACAG CAGCGTCCTG GATTTGGGAG GGAGTGGCAA CCCGTTTGCG  
GTCTGCTGTC GTCGCAGGAC CTAAACCCTC CCTCACCGTT GGGCAAACGC

14601 CACCTTCGCC CCAGGCTGGG GAGAATGTTT TAAAAA AAAAGCATGA  
GTGGAAGCGG GGTCCGACCC CTCTTACAAA ATTTTTTTTT TTTTCGTACT

14651 TGCAAAATAA AAAACTCACC AAGGCCATGG CACCGAGCGT TGGTTTTCTT  
ACGTTTTATT TTTTGAGTGG TTCCGGTACC GTGGCTCGCA ACCAAAAGAA

14701 GTATTCCCTT TAGTATGCGG CGCGCGGCGA TGTATGAGGA AGGTCTCCT  
CATAAGGGGA ATCATAACGC GCGCGCCGCT ACATACTCCT TCCAGGAGGA

14751 CCCTCCTACG AGAGTGTGGT GAGCGCGGCG CCAGTGGCGG CCGCGCTGGG  
GGGAGGATGC TCTCACACCA CTCGCGCCGC GGTACCGCC GCCGCGACCC

14801 TTCTCCCTTC GATGCTCCCC TGGACCCGCC GTTTGTGCCT CCGCGGTACC  
AAGAGGGAAG CTACGAGGGG ACCTGGGCGG CAAACACGGA GGCGCCATGG

14851 TGCGGCCTAC CGGGGGGAGA AACAGCATCC GTTACTCTGA GTTGGCACCC  
ACGCCGATG GCGCCCTCT TTGTCGTAGG CAATGAGACT CAACCGTGGG

14901 CTATTCGACA CCACCCGTGT GTACCTGGTG GACAACAAGT CAACGGATGT  
GATAAGCTGT GGTGGGCACA CATGGACCAC CTGTTGTTCA GTTGCCCTACA

14951 GGCATCCCTG AACTACCAGA ACGACCACAG CAACTTTCTG ACCACGGTCA  
CCGTAGGGAC TTGATGGTCT TGCTGGTGTC GTTGAAAGAC TGGTGCCAGT

15001 TTCAAAACAA TGACTACAGC CCGGGGGAGG CAAGCACACA GACCATCAAT  
AAGTTTTGTT ACTGATGTCG GCGCCCTCC GTTCGTGTGT CTGGTAGTTA

15051 CTTGACGACC GGTGCGACTG GGGCGGCGAC CTGAAAACCA TCCTGCATAC  
GAACCTGCTGG CCAGCGTGAC CCGCGCGCTG GACTTTTGGT AGGACGTATG

15101 CAACATGCCA AATGTGAACG AGTTCATGTT TACCAATAAG TTTAAGGCGC  
GTTGTACGGT TTACACTTGC TCAAGTACAA ATGGTTATTC AAATTCCGCG

Figure 26 P

15151 GGGTGATGGT GTCGCGCTTG CCTACTAAGG ACAATCAGGT GGAGCTGAAA  
CCCACTACCA CAGCGCGAAC GGATGATTCC TGTTAGTCCA CCTCGACTTT

15201 TACGAGTGGG TGGAGTTCAC GCTGCCCCGAG GGCAACTACT CCGAGACCAT  
ATGCTCACCC ACCTCAAGTG CGACGGGCTC CCGTTGATGA GGCTCTGGTA

15251 GACCATAGAC CTTATGAACA ACGCGATCGT GGAGCACTAC TTGAAAGTGG  
CTGGTATCTG GAATACTTGT TGCCTAGCA CCTCGTGATG AACTTTCACC

15301 GCAGACAGAA CGGGGTTCCTG GAAAGCGACA TCGGGGTAAA GTTTGACACC  
CGTCTGTCTT GCCCAAGAC CTTTCGCTGT AGCCCCATTT CAAACTGTGG

15351 CGCAACTTCA GACTGGGGTT TGACCCCGTC ACTGGTCTTG TCATGCCTGG  
GCGTTGAAGT CTGACCCCAA ACTGGGGCAG TGACCAGAAC AGTACGGACC

15401 GGTATATACA AACGAAGCCT TCCATCCAGA CATCATTTTG CTGCCAGGAT  
CCATATATGT TTGCTTCGGA AGGTAGGTCT GTAGTAAAC GACGGTCTTA

15451 GCGGGGTGGA CTTACCCAC AGCCGCCTGA GCAACTTGTT GGGCATCCGC  
CGCCCCACCT GAAGTGGGTG TCGCGGACT CGTTGAACAA CCCGTAGGCG

15501 AAGCGGCAAC CCTTCCAGGA GGGCTTTAGG ATCACCTACG ATGATCTGGA  
TTCGCCGTTG GGAAGGTCCT CCCGAAATCC TAGTGGATGC TACTAGACCT

15551 GGGTGGTAAC ATTCCCGCAC TGTGGATGT GGACGCCTAC CAGGCGAGCT  
CCCACCATG TAAGGGCGTG ACAACCTACA CCTGCGGATG GTCCGCTCGA

15601 TGAAAGATGA CACCGAACAG GCGGGGGTG GCGCAGGCGG CAGCAACAGC  
ACTTTCTACT GTGGCTTGTC CCGCCCCAC CGCGTCCGCC GTCGTTGTCG

15651 AGTGGCAGCG GCGCGGAAGA GAACTCCAAC GCGGCAGCCG CGGCAATGCA  
TCACCGTCGC CGCGCCTTCT CTTGAGGTTG CGCCGTCGGC GCCGTTACGT

15701 GCCGGTGGAG GACATGAACG ATCATGCCAT TCGCGGCGAC ACCTTTGCCA  
CGGCCACCTC CTGTACTTGC TAGTACGGTA AGCGCCGCTG TGGAAACGGT

15751 CACGGGCTGA GGAGAAGCGC GCTGAGGCCG AAGCAGCGGC CGAAGCTGCC  
GTGCCGACT CCTCTTCGCG CGACTCCGGC TTCGTCGCCG GCTTCGACGG

15801 GCCCCCGCTG CGCAACCCGA GGTCGAGAAG CCTCAGAAGA AACC GG TGAT  
CGGGGGCGAC GCGTTGGGCT CCAGCTCTTC GGAGTCTTCT TTGGCCACTA

15851 CAAACCCCTG ACAGAGGACA GCAAGAAACG CAGTTACAAC CTAATAAGCA  
GTTTGGGGAC TGTCTCCTGT CGTTCCTTGC GTCAATGTTG GATTATTCTG

15901 ATGACAGCAC CTTACCCAG TACCGCAGCT GGTACCTTGC ATACAACTAC  
TACTGTCTG GAAGTGGGTC ATGGCGTCGA CCATGGAACG TATGTTGATG

15951 GCGGACCCTC AGACCGGAAT CCGCTCATGG ACCCTGCTTT GCACTCCTGA  
CCGCTGGGAG TCTGGCCTTA GCGGAGTACC TGGGACGAAA CGTGAGGACT

16001 CGTAACCTGC GGCTCGGAGC AGGTCTACTG GTCGTTGCCA GACATGATGC  
GCATTGGACG CCGAGCCTCG TCCAGATGAC CAGCAACGGT CTGTACTACG

16051 AAGACCCCGT GACCTTCCGC TCCACGCGCC AGATCAGCAA CTTTCCGGTG  
TTCTGGGGCA CTGGAAGGCG AGGTGCGCGG TCTAGTCGTT GAAAGGCCAC

Figure 26 Q

16151 GGCCGTCTAC TCCCAACTCA TCCGCCAGTT TACCTCTCTG ACCCACGTGT  
CCGGCAGATG AGGGTTGAGT AGGCGGTCAA ATGGAGAGAC TGGGTGCACA

16201 TCAATCGCTT TCCCGAGAAC CAGATTTTGG CGCGCCCGCC AGCCCCACC  
AGTTAGCGAA AGGGCTCTTG GTCTAAAACC GCGCGGGCGG TCGGGGGTGG

16251 ATCACCACCG TCAGTGAAAA CGTTCCTGCT CTCACAGATC ACGGGACGCT  
TAGTGGTGGC AGTCACTTTT GCAAGGACGA GAGTGTCTAG TGCCCTGCGA

16301 ACCGCTGCGC AACAGCATCG GAGGAGTCCA GCGAGTGACC ATTACTGACG  
TGCGCAGCGG TTGTCTAGC CTCTCAGGT CGCTCACTGG TAATGACTGC

16351 CCAGACGCCG CACCTGCCCC TACGTTTACA AGGCCCTGGG CATAGTCTCG  
GGTCTGCGGC GTGGACGGGG ATGCAAATGT TCCGGGACCC GTATCAGAGC

16401 CCGCGCGTCC TATCGAGCCG CACTTTTGA GCAAGCATGT CCATCCTTAT  
GGCGCGCAGG ATAGCTCGGC GTGAAAACT CGTTCTGTACA GGTAGGAATA

16451 ATCGCCAGC AATAACACAG GCTGGGGCCT GCGCTTCCCA AGCAAGATGT  
TAGCGGGTCG TTATTGTGTC CGACCCCGGA CGCGAAGGGT TCGTTCTACA

16501 TTGGCGGGGC CAAGAAGCGC TCCGACCAAC ACCCAGTGCG CGTGCGCGGG  
AACCGCCCCG GTTCTTCGCG AGGCTGGTTG TGGGTCACGC GCACGCGCCC

16551 CACTACCGCG CGCCCTGGGG CGCGCACAAA CGCGGCCGCA CTGGGCGCAC  
GTGATGGCGC GCGGGACCCC GCGCGTGTTT GCGCCGGCGT GACCCGCGTG

16601 CACCGTCGAT GACGCCATCG ACGCGGTGGT GGAGGAGGCG CGCAACTACA  
GTGGCAGCTA CTGCGGTAGC TGCGCCACCA CCTCCTCCGC GCGTTGATGT

16651 CGCCACGCC GCCACAGTG TCCACAGTGG ACGCGGCCAT TCAGACCGTG  
GCGGGTGCGG CGGTGGTCAC AGGTGTCACC TGCGCCGGTA AGTCTGGCAC

16701 GTGCGCGGAG CCCGGCGCTA TGCTAAAATG AAGAGACGGC GGAGGCGCGT  
CACGCGCCTC GGGCCGCGAT ACGATTTTAC TTCTCTGCCG CCTCCGCGCA

16751 AGCACGTGCG CACCGCCGCC GACCCGGCAC TGCCGCCCAA CGCGCGCGCG  
TCGTGCAGCG GTGGCGGCGG CTGGGCCGTG ACGGCGGGTT GCGCGCCGCC

16801 CGGCCCTGCT TAACCGCGCA CGTCGCACCG GCCGACGGGC GGCCATGCGG  
GCCGGGACGA ATTGGCGCGT GCAGCGTGGC CGGCTGCCCG CCGGTACGCC

16851 GCCGCTCGAA GGCTGGCCGC GGGTATTGTC ACTGTGCCCC CCAGGTCCAG  
CGGCAGGCTT CCGACCGGCG CCCATAACAG TGACACGGGG GGTCCAGGTC

16901 GCGACGAGCG GCCGCCGAG CAGCCGCGGC CATTAGTGCT ATGACTCAGG  
CGCTGCTCGC CGGCGGCGTC GTCGGCGCCG GTAATCACGA TACTGAGTCC

16951 GTCGCAGGGG CAACGTGTAT TGGGTGCGCG ACTCGGTTAG CGGCCTGCGC  
CAGCGTCCCC GTTGACATA ACCCACGCGC TGAGCCAATC GCCGGACGCG

17001 GTGCCCCTGC GCACCCGCCC CCCGCGCAAC TAGATTGCAA GAAAAAATA  
CACGGGCACG CGTGGGCGGG GGGCGCGTTG ATCTAACGTT CTTTTTTGAT

Figure 26 R



17101 CTATGTCCAA GCGCAAAATC AAAGAAGAGA TGCTCCAGGT CATCGCGCCG  
GATACAGGTT CGCGTTTATG TTTCTTCTCT ACGAGGTCCA GTAGCGCGGC

17151 GAGATCTATG GCGGGGCGAA GAAGGAAGAG CAGGATTACA AGCGGGGAAA  
CTCTAGATAC CGGGGGGCTT CTTCCTTCTC GTCCTAATGT TCGGGGCTTT

17201 GCTAAAGCGG GTCAAAAAGA AAAAGAAAGA TGATGATGAT GAAC TTGACG  
CGATTTTCGCC CAGTTTCTCT TTTCTTCTCT ACTACTACTA CTTGAACTGC

17251 ACGAGGTGGA ACTGCTGCAC GCTACCGCGC CCAGGCGACG GGTACAGTGG  
TGCTCCACCT TGACGACGTG CGATGGCGCG GGTCCGCTGC CCATGTCACC

17301 AAAGGTCGAC GCGTAAAACG TGT TTTGCGA CCCGGCACCA CCGTAGTCTT  
TTTCCAGCTG CGCATTTTGC ACAAACGCT GGGCCGTGGT GGCATCAGAA

17351 TACGCCCCGT GAGCGCTCCA CCCGCACCTA CAAGCGCGTG TATGATGAGG  
ATCGGGGCCA CTCGCGAGGT GGGCGTGGAT GTTCGCGCAC ATACTACTCC

17401 TGTACGGCGA CGAGGACCTG CTTGAGCAGG CCAACGAGCG CCTCGGGGAG  
ACATGCCGCT GTCCTGGAC GAACTCGTCC GGTGCTCGC GGAGCCCCCTC

17451 TTTGCCTACG GAAAGCGGCA TAAGGACATG CTGGCGTTGC CGCTGGACGA  
AAACGGATGC CTTTCGCGGT ATTCTGTAC GACCGCAACG GCGACCTGCT

17501 GGGCAACCCA ACACCTAGCC TAAAGCCCGT AACACTGCAG CAGGTGCTGC  
CCCCTTGGGT TGTGGATCGG ATTTGGGCA TTGTGACGTC GTCCACGACG

17551 CCGCGCTTGC ACCGTCCGAA GAAAAGCGCG GCCTAAAGCG CGAGTCTGGT  
GGCGCGAACG TGGCAGGCTT CTTTTCGCGC CGGATTTGCG GCTCAGACCA

17601 GACTTGGCAC CCACCGTGCA GCTGATGGTA CCCAAGCGCC AGCGACTGGA  
CTGAACCGTG GGTGGCACGT CGACTACCAT GGGTTCGCGG TCGCTGACCT

17651 AGATGTCTTG GAAAAAATGA CCGTGGAACC TGGGCTGGAG CCCGAGGTCC  
TCTACAGAAC CTTTTTACT GGCACCTTGG ACCCGACCTC GGGCTCCAGG

17701 GCGTGCGGCC AATCAAGCAG GTGGCGCCGG GACTGGGCGT GCAGACCGTG  
CGCACGCCGG TTAGTTCGTC CACCGCGGCC CTGACCCGCA CGTCTGGCAC

17751 GACGTTTCTG TACCCACTAC CAGTAGCACC AGTATTGCCA CCGCCACAGA  
CTGCAAGTCT ATGGGTGATG GTCATCGTGG TCATAACGGT GCGGGTGTCT

17801 GGGCATGGAG ACACAAACGT CCCCCTTGC CTCAGCGGTG GCGGATGCCG  
CCCGTACCTC TGTGTTTGCA GGGGCCAACG GAGTCGCCAC CGCCTACGGC

17851 CGGTGCAGGC GGTGCTGCG GCGCGTCCA AGACCTCTAC GGAGGTGCAA  
GCCACGTCCG CCAGCGACGC CGGCGCAGGT TCTGGAGATG CCTCCACGTT

17901 ACGGACCCGT GGATGTTTCG CGTTTCAGCC CCCCAGCGCC CGCGCCGTTT  
TGCTTGGGCA CCTACAAAGC GCAAAGTCGG GGGGCCGCGG GCGCGGCAAG

17951 GAGGAAGTAC GCGCGGCCA GCGCGCTACT GCGGAATAT GCCCTACATC  
CTCCTTCATG CCGCGGCGGT CGCGCGATGA CGGGCTTATA CGGGATGTAG

Figure 26S

18051 AGACGAGCAA CTACCCGACG CCGAACCACC ACTGGAACCC GCCGCCGCCG  
TCTGCTCGTT GATGGGCTGC GGCTTGTTGG TGACCTTGGG CGGCGGCGGC

18101 TCGCCGTCGC CAGCCCGTGC TGGCCCCGAT TTCCGTGCGC AGGGTGGCTC  
AGCGGCAGCG GTCGGGCACG ACCGGGGCTA AAGGCACGCG TCCCACCGAG

18151 GCGAAGGAGG CAGGACCCTG GTGCTGCCAA CAGCGCGCTA CCACCCAGC  
CGCTTCTCTC GTCCTGGGAC CACGACGGTT GTCGCGCGAT GGTGGGGTGC

18201 ATCGTTTAAA AGCCGGTCTT TGTGGTTCTT GCAGATATGG CCCTCACCTG  
TAGCAAATTT TCGGCCAGAA ACACCAAGAA CGTCTATACC GGGAGTGGAC

18251 CCGCCTCCGT TTCCCGGTGC CGGGATTCCG AGGAAGAATG CACCGTAGGA  
GGCGGAGGCA AAGGGCCACG GCCCTAAGGC TCCTTCTTAC GTGGCATCCT

18301 GGGGCATGGC CGGCCACGGC CTGACGGGCG GCATGCGTCG TGCGCACCAC  
CCCCGTACCG GCCGGTGCCG GACTGCCCGC CGTACGCAGC ACGCGTGGTG

18351 CGGCGGCGGC GCGCGTCGCA CCGTCGCATG CGCGGCGGTA TCCTGCCCTT  
GCCGCCGCCG CCGCGACGCT GGCAGCGTAC GCGCCGCCAT AGGACGGGGA

18401 CCTTATTCCA CTGATCGCCG CGGCGATTGG CGCCGTGCCC GGAATTGCAT  
GGAATAAGGT GACTAGCGGC GCCGCTAACC GCGGCACGGG CCTTAACGTA

18451 CCGTGGCCTT GCAGGCGCAG AGACACTGAT TAAAAACAAG TTGCATGTGG  
GGCACCGGAA CGTCCGCGTC TCTGTGACTA ATTTTGTTC AACGTACACC

18501 AAAAAACAAA ATAAAAAGTC TGGACTCTCA CGCTCGCTTG GTCCTGTAAC  
TTTTTAGTTT TATTTTTCAG ACCTGAGAGT GCGAGCGAAC CAGGACATTG

18551 TATTTGTAG AATGGAAGAC ATCAACTTTG CGTCTCTGGC CCCGCGACAC  
ATAAACATC TTACCTTCTG TAGTTGAAAC GCAGAGACCG GGGCGCTGTG

18601 GGCTCGCGCC CGTTCATGGG AAACCTGGCA GATATCGGCA CCAGCAATAT  
CCGAGCGCGG GCAAGTACCC TTTGACCGTT CTATAGCCGT GGTGCTTATA

18651 GAGCGGTGGC GCCTTCAGCT GGGGCTCGCT GTGGAGCGGC ATTAATAATT  
CTCGCCACCG CGGAAGTCGA CCCCAGCGCA CACCTCGCCG TAATTTTAA

18701 TCGGTTCAC CGTTAAGAAC TATGGCAGCA AGGCCTGGAA CAGCAGCACA  
AGCCAAGGTG GCAATTCTTG ATACCGTCGT TCCGGACCTT GTCGTCGTGT

18751 GGCCAGATGC TGAGGGATAA GTTGAAAGAG CAAAATTTC AACAAAAGGT  
CCGGTCTACG ACTCCCTATT CAACTTTCTC GTTTTAAAGG TTGTTTTCCA

18801 GGTAGATGGC CTGGCCTCTG GCATTAGCGG GGTGGTGGAC CTGGCCAACC  
CCATCTACCG GACCGGAGAC CGTAATCGCC CCACCACCTG GACCGGTGG

18851 AGGCAGTGCA AAATAAGATT AACAGTAAGC TTGATCCCCG CCCTCCCGTA  
TCCGTACAGT TTTATTCTAA TTGTCATTG AACTAGGGGC GGGAGGGCAT

18901 GAGGAGCCTC CACCGGCCGT GGAGACAGTG TCTCCAGAGG GCGGTGGCGA  
CTCCTCGGAG GTGGCCGCA CCTCTGTAC AGAGGTCTC CCGCACCGCT

Figure 26 T

19001 AGCCTCCCTC GTACGAGGAG GCACTAAAGC AAGGCCTGCC CACCACCCGT  
TCGGAGGGAG CATGCTCCTC CGTGATTTCG TTCCGGACGG GTGGTGGGCA

19051 CCCATCGCGC CCATGGCTAC CGGAGTGCTG GGCCAGCACA CCCCCGTAAC  
GGGTAGCGCG GGTACCGATG GCCTCACGAC CCGGTCGTGT GTGGGCATTG

19101 GCTGGACCTG CCTCCCCCG CCGACACCCA GCAGAAACCT GTGCTGCCAG  
CGACCTGGAC GGAGGGGGGC GGCTGTGGGT CGTCTTTGGA CACGACGGTC

19151 GCCCGACCGC CGTTGTTGTA ACCCGTCCTA GCCGCGCGTC CCTGCGCCGC  
CGGGCTGGCG GCAACAACAT TGGGCAGGAT CGGCGCGCAG GGACGCGGCG

19201 GCCGCCAGCG GTCCGCGATC GTTGGGCCCC GTAGCCAGTG GCAACTGGCA  
CGGCGGTGCG CAGGCGCTAG CAACGCCGGG CATCGGTCAC CGTTGACCGT

19251 AAGCACACTG AACAGCATCG TGGGTCTGGG GGTGCAATCC CTGAAGCGCC  
TTCGTGTGAC TTGTCGTAGC ACCCAGACCC CCACGTTAGG GACTTCGCGG

19301 GACGATGCTT CTGATAGCTA ACGTGTCGTA TGTGTGTCAT GTATGCGTCC  
CTGTACGAA GACTATCGAT TGCACAGCAT ACACACAGTA CATACGCAGG

19351 ATGTCGCCGC CAGAGGAGCT GCTGAGCCGC CGCGCGCCCG CTTTCCAAGA  
TACAGCGGCG GTCTCCTCGA CGACTCGGCG GCGCGCGGGC GAAAGGTTCT

19401 TGGCTACCCC TTCGATGATG CCGCAGTGGT CTTACATGCA CATCTCGGGC  
ACCGATGGGG AAGCTACTAC GCGGTCACCA GAATGTACGT GTAGAGCCCC

19451 CAGGACGCCT CGGAGTACCT GAGCCCCGGG CTGGTGCACT TTGCCCCGCG  
GTCTGCGGA GCCTCATGGA CTCGGGGCCC GACCACGTCA AACGGGCGCG

19501 CACCGAGACG TACTTCAGCC TGAATAACAA GTTTAGAAAC CCCACGGTGG  
GTGGCTCTGC ATGAAGTCGG ACTTATTGTT CAAATCTTTG GGGTGCCACC

19551 CGCCTACGCA CGACGTGACC ACAGACCGGT CCCAGCGTTT GACGCTGCGG  
GCGGATGCGT GCTGCACTGG TGTCTGGCCA GGGTCGCAA CTGCGACGCC

19601 TTCATCCCTG TGGACCGTGA GGATACTGCG TACTCGTACA AGGCGCGGTT  
AAGTAGGGAC ACCTGGCACT CCTATGACGC ATGAGCATGT TCCGCGCCAA

19651 CACCCTAGCT GTGGGTGATA ACCGTGTGCT GGACATGGCT TCCACGTACT  
GTGGGATCGA CACCCACTAT TGGCACACGA CCTGTACCGA AGGTGCATGA

19701 TTGACATCCG CGGCGTGCTG GACAGGGGCC CTACTTTTAA GCCCTACTCT  
AACTGTAGGC GCCGCACGAC CTGTCCCCGG GATGAAAATT CGGGATGAGA

19751 GGCACCTGCCT ACAACGCCCT GGCTCCCAAG GGTGCCCCAA ATCCTTGCGA  
CCGTGACGGA TGTTCGCGGA CCGAGGGTTC CCACGGGGTT TAGGAACGCT

19801 ATGGGATGAA GCTGCTACTG CTCTTGAAAT AAACCTAGAA GAAGAGGACG  
TACCCTACTT CGACGATGAC GAGAACTTTA TTTGGATCTT CTTCTCCTGC

19851 ATGACAACGA AGACGAAGTA GACGAGCAAG CTGAGCAGCA AAAAACTCAC  
TACTGTTGCT TCTGCTTCAT CTGCTCGTTC GACTCGTCGT TTTTGTAGTG

Figure 26 u

19951	TCAAATAGGT	GTCGAAGGTC	AAACACCTAA	ATATGCCGAT	AAAACATTTT
	AGTTTATCCA	CAGCTTCCAG	TTTGTGGATT	TATACGGCTA	TTTTGTAAAG
20001	AACCTGAACC	TCAAATAGGA	GAATCTCAGT	GGTACGAAAC	AGAAATTAAT
	TTGGACTTGG	AGTTTATCCT	CTTAGAGTCA	CCATGCTTTG	TCTTTAATTA
20051	CATGCAGCTG	GGAGAGTCCT	AAAAAAGACT	ACCCCAATGA	AACCATGTTA
	GTACGTCGAC	CCTCTCAGGA	TTTTTCTGA	TGGGGTACT	TTGGTACAAT
20101	CGGTTCATAT	GCAAAACCCA	CAAATGAAAA	TGGAGGGCAA	GGCATTCTTG
	GCCAAGTATA	CGTTTTGGGT	GTTTACTTTT	ACCTCCCGTT	CCGTAAGAAC
20151	TAAAGCAACA	AAATGGAAAG	CTAGAAAGTC	AAGTGGAAAT	GCAATTTTTT
	ATTTCTGTGT	TTTACCTTTC	GATCTTTCAG	TTACCTTTTA	CGTTAAAAAG
20201	TCAACTACTG	AGGCAGCCGC	AGGCAATGGT	GATAACTTGA	CTCCTAAAGT
	AGTTGATGAC	TCCGTCGGCG	TCCGTTACCA	CTATTGAAC	GAGGATTTCA
20251	GGTATTGTAC	AGTGAAGATG	TAGATATAGA	AACCCAGAC	ACTCATATTT
	CCATAACATG	TCACTTCTAC	ATCTATATCT	TTGGGGTCTG	TGAGTATAAA
20301	CTTACATGCC	CACTATTAAAG	GAAGGTAAC	CACGAGAACT	AATGGGCCAA
	GAATGTACGG	GTGATAATTC	CTTCCATTGA	GTGCTCTTGA	TTACCCGGTT
20351	CAATCTATGC	CCAACAGGCC	TAATTACATT	GCTTTTAGGG	ACAATTTTAT
	GTTAGATACG	GGTTGTCCGG	ATTAATGTAA	CGAAAATCCC	TGTTAAAATA
20401	TGGTCTAATG	TATTACAACA	GCACGGGTAA	TATGGGTGTT	CTGGCGGGCC
	ACCAGATTAC	ATAATGTTGT	CGTGCCCAT	ATACCCACAA	GACCGCCCGG
20451	AAGCATCGCA	GTTGAATGCT	GTTGTAGATT	TGCAAGACAG	AAACACAGAG
	TTCGTAGCGT	CAACTTACGA	CAACATCTAA	ACGTTCTGTC	TTTGTGTCTC
20501	CTTTCATACC	AGCTTTTGCT	TGATTCCATT	GGTGATAGAA	CCAGGTACTT
	GAAAGTATGG	TCGAAAACGA	ACTAAGGTAA	CCACTATCTT	GGTCCATGAA
20551	TTCTATGTGG	AATCAGGCTG	TTGACAGCTA	TGATCCAGAT	GTTAGAATTA
	AAGATACACC	TTAGTCCGAC	AACTGTCGAT	ACTAGGTCTA	CAATCTTAAT
20601	TTGAAATCA	TGGAACGTAA	GATGAACTTC	CAAATTACTG	CTTTCCACTG
	AACTTTTAGT	ACCTTGACTT	CTACTTGAAG	GTTTAATGAC	GAAAGGTGAC
20651	GGAGGTGTGA	TTAATACAGA	GACTCTTACC	AAGGTAAAAC	CTAAAACAGG
	CCTCCACACT	AATTATGTCT	CTGAGAATGG	TTCCATTTTG	GATTTTGTCC
20701	TCAGGAAAAT	GGATGGGAAA	AAGATGCTAC	AGAATTTTCA	GATAAAAATG
	AGTCCTTTTA	CCTACCCTTT	TTCTACGATG	TCTTAAAAGT	CTATTTTAC
20751	AAATAAGAGT	TGGAAATAAT	TTTGCCATGG	AAATCAATCT	AAATGCCAAC
	TTTATTCTCA	ACCTTTATTA	AAACGGTACC	TTTAGTTAGA	TTTACGGTTG
20801	CTGTGGAGAA	ATTTCTGTGA	CTCCAACATA	GCGCTGTATT	TGCCCCGACAA
	GACACCTCTT	TAAAGGACAT	GAGGTTGTAT	CGCGACATAA	ACGGGCTGTT

Figure 26 v

20901 ACGACTACAT GAACAAGCGA GTGGTGGCTC CCGGGCTAGT GGACTGCTAC  
TGCTGATGTA CTTGTTGCT CACCACCGAG GGCCCGATCA CCTGACGATG

20951 ATTAACCTTG GAGCACGCTG GTCCCTTGAC TATATGGACA ACGTCAACCC  
TAATTGGAAC CTCGTGCGAC CAGGGAAGTG ATATACCTGT TGCAGTTGGG

21001 ATTTAACCAC CACCGCAATG CTGGCCTGCG CTACCGCTCA ATGTTGCTGG  
TAAATTGGTG GTGGCGTTAC GACCGGACGC GATGGCGAGT TACAACGACC

21051 GCAATGGTGC CTATGTGCCC TTCCACATCC AGGTGCCTCA GAAGTTCTTT  
CGTTACCAGC GATACACGGG AAGGTGTAGG TCCACGGAGT CTTCAAGAAA

21101 GCCATTAAAA ACCTCCTTCT CCTGCCGGGC TCATACACCT ACGAGTGGAA  
CGGTAATTTT TGGAGGAAGA GGACGGCCCG AGTATGTGGA TGCTCACCTT

21151 CTTCAGGAAG GATGTTAACA TGGTTCTGCA GAGCTCCCTA GGAAATGACC  
GAAGTCCTTC CTACAATTGT ACCAAGACGT CTCGAGGGAT CCTTTACTGG

21201 TAAGGGTTGA CGGAGCCAGC ATTAAGTTTG ATAGCATTTG CCTTTACGCC  
ATTCCCAACT GCCTCGGTCG TAATTCAAAC TATCGTAAAC GGAAATGCGG

21251 ACCTTCTTCC CCATGGCCCA CAACACCGCC TCCACGCTTG AGGCCATGCT  
TGGAAGAAGG GGTACCGGGT GTTGTGGCGG AGGTGCGAAC TCCGGTACGA

21301 TAGAAACGAC ACCAACGACC AGTCCTTTAA CGACTATCTC TCCGCCGCCA  
ATCTTTGCTG TGGTTGCTGG TCAGGAAATT GCTGATAGAG AGGCGGCGGT

21351 ACATGCTCTA CCCTATACCC GCCAACGCTA CCAACGTGCC CATATCCATC  
TGTACGAGAT GGGATATGGG CGGTTGCGAT GGTGTCACGG GTATAGGTAG

21401 CCCTCCCGCA ACTGGGCGGC TTTCCGCGGC TGGGCCTTCA CGCGCCTTAA  
GGGAGGGCGT TGACCCGCCG AAAGGCGCCG ACCCGGAAGT GCGCGGAATT

21451 GACTAAGGAA ACCCCATCAC TGGGCTCGGG CTACGACCCT TATTACACCT  
CTGATTCTT TGGGGTAGTG ACCCGAGCCC GATGCTGGGA ATAATGTGGA

21501 ACTCTGGCTC TATACCCTAC CTAGATGGAA CCTTTTACCT CAACCACACC  
TGAGACCGAG ATATGGGATG GATCTACCTT GGAAATGGA GTTGGTGTGG

21551 TTTAAGAAGG TGGCCATTAC CTTTGACTCT TCTGTCAGCT GGCCTGGCAA  
AAATTCTTCC ACCGGTAATG GAAACTGAGA AGACAGTCGA CCGGACCGTT

21601 TGACCGCCTG CTTACCCCA ACGAGTTTGA AATTAAGCGC TCAGTTGACG  
ACTGGCGGAC GAATGGGGGT TGCTCAAAC TTAATTCGCG AGTCAACTGC

21651 GGGAGGGTTA CAACGTTGCC CAGTGTAACA TGACCAAAGA CTGGTTCCTG  
CCCTCCCAAT GTTGCAACGG GTCACATTGT ACTGGTTTCT GACCAAGGAC

21701 GTACAAATGC TAGCTAACTA TAACATTGGC TACCAGGGCT TCTATATCCC  
CATGTTTACG ATCGATTGAT ATTGTAACCG ATGGTCCCGA AGATATAGGG

21751 AGAGAGCTAC AAGGACCGCA TGTACTCCTT CTTTAGAAAC TTCCAGCCCA  
TCTCTCGATG TTCCTGGCGT ACATGAGGAA GAAATCTTTG AAGGTCGGGT

Figure 26 W

21851 GGCATCCTAC ACCAACACAA CAACTCTGGA TTTGTTGGCT ACCTTGCCCC  
CCGTAGGATG TGGTTGTGTT GTTGAGACCT AAACAACCGA TGAACGGGG

21901 CACCATGCGC GAAGGACAGG CCTACCCTGC TAACTTCCCC TATCCGCTTA  
GTGGTACGCG CTTCTGTCC GGATGGGACG ATTGAAGGGG ATAGGCGAAT

21951 TAGGCAAGAC CGCAGTTGAC AGCATTACCC AGAAAAAGTT TCTTTGCGAT  
ATCCGTTCTG GCGTCAACTG TCGTAATGGG TCTTTTTCAA AGAAACGCTA

22001 CGCACCTTT GCGCATCCC ATTCTCCAGT AACTTTATGT CCATGGGCGC  
GCGTGGGAAA CCGCGTAGGG TAAGAGGTCA TTGAAATACA GGTACCCGCG

22051 ACTCACAGAC CTGGGCCAAA ACCTTCTCTA CGCCAACCTC GCCCACGCGC  
TGAGTGTCTG GACCCGGTTT TGAAGAGAT GCGGTTGAGG CGGGTGCGCG

22101 TAGACATGAC TTTTGAGGTG GATCCCATGG ACGAGCCCAC CCTTCTTTAT  
ATCTGTACTG AAAACTCCAC CTAGGTACC TGCTCGGGTG GGAAGAAATA

22151 GTTTTGTGTTG AAGTCTTTGA CGTGGTCCGT GTGCACCAGC CGCACCGCGG  
CAAAACAAAC TTCAGAACT GCACCAGGCA CACGTGGTCG GCGTGGCGCC

22201 CGTCATCGAA ACCGTGTACC TGCGCACGCC CTTCTCGGCC GGCAACGCCA  
GCAGTAGCTT TGGCACATGG ACGCGTGC GG GAAGAGCCGG CCGTTGCGGT

22251 CAACATAAAG AAGCAAGCAA CATCAACAAC AGCTGCCGCC ATGGGCTCCA  
GTTGTATTC TTCGTTCTGTT GTAGTTGTTG TCGACGGCGG TACCCGAGGT

22301 GTGAGCAGGA ACTGAAAGCC ATTGTCAAAG ATCTTGTTG TGGGCCATAT  
CACTCGTCCT TGACTTTCGG TAACAGTTTC TAGAACCAAC ACCCGGTATA

22351 TTTTTGGGCA CCTATGACAA GCGCTTTCCA GGCTTTGTTT CTCCACACAA  
AAAAACCCGT GGATACTGTT CGCGAAAGGT CCGAAACAAA GAGGTGTGTT

22401 GCTCGCCTGC GCCATAGTCA ATACGGCCGG TCGCGAGACT GGGGCGTAC  
CGAGCGGACG CCGTATCAGT TATGCCGGCC AGCGCTCTGA CCCCCGCATG

22451 ACTGGATGGC CTTTGCCTGG AACCCGCACT CAAAAACATG CTACCTCTTT  
TGACCTACCG GAAACGGACC TTGGGCGTGA GTTTTGTAC GATGGAGAAA

22501 GAGCCCTTTG GCTTTTCTGA CCAGCGACTC AAGCAGGTTT ACCAGTTTGA  
CTCGGGAAAC CGAAAAGACT GGTGCTGAG TTCGTCCAAA TGGTCAAACT

22551 GTACGAGTCA CTCCTGCGCC GTAGCGCCAT TGCTTCTTCC CCCGACCGCT  
CATGCTCAGT GAGGACGCGG CATCGCGGTA ACGAAGAAGG GGGCTGGCGA

22601 GTATAACGCT GGAAAAGTCC ACCCAAAGCG TACAGGGGCC CAACTCGGCC  
CATATTGCGA CCTTTTCAGG TGGGTTTCGC ATGTCCCCGG GTTGAGCCGG

22651 GCCTGTGGAC TATTCTGCTG CATGTTTCTC CACGCCTTTG CCAACTGGCC  
CGGACACCTG ATAAGACGAC GTACAAAGAG GTGCGGAAAC GGTGACCGG

22701 CCAAACCTCC ATGGATCACA ACCCCACCAT GAACCTTATT ACCGGGGTAC  
GGTTTGAGGG TACCTAGTGT TGGGGTGGTA CTTGGAATAA TGGCCCCATG

Figure 26 x

22801 CAGGAACAGC TCTACAGCTT CCTGGAGCGC CACTCGCCC ACTTCGCGAG  
 GTCTTTGTCT AGATGTCGAA GGACCTCGCG GTGAGCGGGA TGAAGGCGTC

22851 CCACAGTGCG CAGATTAGGA GCGCCACTTC TTTTGTGCAC TTGAAAAACA  
 GGTGTCACGC GTCTAATCCT CGCGGTGAAG AAAACAGTG AACTTTTTGT

22901 TGTAATAATA ATGTACTAGA GACACTTTCA ATAAAGGCAA ATGCTTTTAT  
 ACATTTTTAT TACATGATCT CTGTGAAAGT TATTTCGGT TACGAAATA

22951 TTGTACACTC TCGGGTGATT ATTTACCCCC ACCCTTGCCG TCTGCGCCGT  
 AACATGTGAG AGCCCACTAA TAAATGGGGG TGGGAACGCG AGACGCGGCA

23001 TTAAAAATCA AAGGGTCTT GCGCGCATC GCTATGCGCC ACTGGCAGGG  
 AATTTTTAGT TTCCCCAAGA CGGCGCGTAG CGATACGCGG TGACCGTCCC

23051 ACACGTTGCG ATACTGGTGT TTAGTGCTCC ACTTAACTC AGGCACAACC  
 TGTGCAACGC TATGACCACA AATCACGAGG TGAATTTGAG TCCGTGTTGG

23101 ATCCGCGGCA GCTCGGTGAA GTTTTCACTC CACAGGCTGC GCACCATCAC  
 TAGGCGCCGT CGAGCCACTT CAAAAGTGAG GTGTCCGACG CGTGGTAGTG

23151 CAACGCGTTT AGCAGGTGCG GCGCCGATAT CTTGAAGTCG CAGTTGGGGC  
 GTTGCAGAAA TCGTCCAGCC CGCGGCTATA GAACTTCAGC GTCAACCCCG

23201 CTCCGCCCTG CGCGCGGAG TTGCGATACA CAGGGTTGCA GCACTGGAAC  
 GAGGCGGGAC GCGCGCGCTC AACGCTATGT GTCCCAACGT CGTGACCTTG

23251 ACTATCAGCG CCGGGTGGTG CACGCTGGCC AGCACGCTCT TGTCGGAGAT  
 TGATAGTCGC GGCCACCAC GTGCGACCGG TCGTGCGAGA ACAGCCTCTA

23301 CAGATCCGCG TCCAGGTCCT CCGCGTTGCT CAGGGCGAAC GGAGTCAACT  
 GTCTAGGCGC AGGTCCAGGA GGCGCAACGA GTCCCGCTTG CCTCAGTTGA

23351 TTGGTAGCTG CTTTCCAAA AAGGGCGCGT GCCCAGGCTT TGAGTTGCAC  
 AACCATCGAC GGAAGGGTTT TTCCCGCGCA CGGGTCCGAA ACTCAACGTG

23401 TCGCACCGTA GTGGCATCAA AAGGTGACCG TGCCCGGTCT GGGCGTTAGG  
 AGCGTGGCAT CACCGTAGTT TTCCACTGGC ACGGGCCAGA CCCGCAATCC

23451 ATACAGCGCC TGCATAAAAG CCTTGATCTG CTTAAAAGCC ACCTGAGCCT  
 TATGTCGCGG ACGTATTTTC GGAAC TAGAC GAATTTTCGG TGGACTCGGA

23501 TTGCGCCTTC AGAGAAGAAC ATGCCGCAAG ACTTGCCGGA AAAGTATTG  
 AACGCGGAAG TCTCTTCTTG TACGGCGTTC TGAACGGCCT TTTGACTAAC

23551 GCCGGACAGG CCGCGTCGTG CACGCAGCAC CTTGCGTCGG TGTTGGAGAT  
 CGGCCTGTCC GCGCGAGCAC GTGCGTCGTG GAACGCAGCC ACAACCTCTA

23601 CTGCACCACA TTTCGGCCCC ACCGGTTCTT CACGATCTTG GCCTTGCTAG  
 GACGTGGTGT AAAGCCGGGG TGGCCAAGAA GTGCTAGAAC CGGAACGATC

23651 ACTGCTCCTT CAGCGCGCGC TGCCCGTTTT CGCTCGTCAC ATCCATTTCA  
 TGACAGGAA GTCGCGCGCG ACGGGCAAAA GCGAGCAGTG TAGGTAAAGT

Figure 26 Y

23701 ATCACGTGCT CTTATTAT CATAATGCTT CCGTGTAGAC ACTTAAGCTC  
TAGTGCACGA GGAATAAATA GTATTACGAA GGCACATCTG TGAATTCGAG

23751 GCCTTCGATC TCAGCGCAGC GGTGCAGCCA CAACGCGCAG CCCGTGGGCT  
CGGAAGCTAG AGTCGCGTCG CCACGTCGGT GTTGC GCGTC GGGCACCCGA

23801 CGTGATGCTT GTAGGTCACC TCTGCAAACG ACTGCAGGTA CGCCTGCAGG  
GCACTACGAA CATCCAGTGG AGACGTTTGC TGACGTCCAT GCGGACGTCC

23851 AATCGCCCCA TCATCGTCAC AAAGGTCTTG TTGCTGGTGA AGGTCAGCTG  
TTAGCGGGGT AGTAGCAGTG TTTCCAGAAC AACGACCACT TCCAGTCGAC

23901 CAACCCGCGG TGCTCCTCGT TCAGCCAGGT CTTGCATACG GCCGCCAGAG  
GTTGGGCGCC ACGAGGAGCA AGTCGGTCCA GAACGTATGC CGGCGGTCTC

23951 CTTCCACTTG GTCAGGCAGT AGTTTGAAGT TCGCCTTTAG ATCGTTATCC  
GAAGGTGAAC CAGTCCGTCA TCAAACCTCA AGCGGAAATC TAGCAATAGG

24001 ACGTGGTACT TGTCCATCAG CGCGCGCGCA GCCTCCATGC CCTTCTCCCA  
TGCAACATGA ACAGGTAGTC GCGCGCGCGT CGGAGGTACG GGAAGAGGGT

24051 CGCAGACACG ATCGGCACAC TCAGCGGGTT CATCACCGTA ATTTCACTTT  
GCGTCTGTGC TAGCCGTGTG AGTCGCCAA GTAGTGGCAT TAAAGTGAAA

24101 CCGCTTCGCT GGGCTCTTCC TCTTCCTCTT GCGTCCGCAT ACCACGCGCC  
GGCGAAGCGA CCCGAGAAGG AGAAGGAGAA CGCAGGCGTA TGGTGC GCGG

24151 ACTGGGTCGT CTTCAATCAG CCGCCGCACT GTGCGCTTAC CTCCTTTGCC  
TGACCCAGCA GAAGTAAGTC GGC GCGGTGA CACGCGAATG GAGGAAACGG

24201 ATGCTTGATT AGCACCGGTG GGTGCTGAA ACCCACCATT TGTAGCGCCA  
TACGAATAA TCGTGGCCAC CCAACGACTT TGGGTGGTAA ACATCGCGGT

24251 CATCTTCTCT TTCTTCCTCG CTGTCCACGA TTACCTCTGG TGATGGCGGG  
GTAGAAGAGA AAGAAGGAGC GACAGGTGCT AATGGAGACC ACTACCGCCC

24301 CGCTCGGGCT TGGGAGAAGG GCGCTTCTTT TTCTTCTTGG GCGCAATGGC  
GCGAGCCCGA ACCCTCTTCC CGCGAAGAAA AAGAAGAACC CGCGTTACCG

24351 CAAATCCGCC GCCGAGGTG ATGGCCGCGG GCTGGGTGTG CGCGGCACCA  
GTTTAGGCGG CGGCTCCAGC TACCGGCGCC CGACCCACAC GCGCCGTGGT

24401 GCGCGTCTTG TGATGAGTCT TCCTCGTCCT CGGACTCGAT ACGCGCGCTC  
CGCGCAGAAC ACTACTCAGA AGGAGCAGGA GCCTGAGCTA TGCGGCGGAG

24451 ATCCGCTTTT TTGGGGGCGC CCGGGGAGGC GCGGGCGACG GGGACGGGGA  
TAGGCGAAAA AACCCCGCG GGGCCCTCCG CCGCCGCTGC CCCTGCCCCT

24501 CGACACGTCC TCCATGGTTG GGGGACGTG CGCCGCACCG CGTCCGCGCT  
GCTGTGCAGG AGGTACCAAC CCCCTGCAGC GCGGCGTGGC GCAGGCGCGA

24551 CGGGGGTGGT TTCGCGCTGC TCCTCTTCCC GACTGGCCAT TTCCTTCTCC  
GCCCCACCA AAGCGCGACG AGGAGAAGGG CTGACCGGTA AAGGAAGAGG

24601 TATAGGCAGA AAAAGATCAT GGAGTCAGTC GAGAAGAAGG ACAGCCTAAC  
ATATCCGTCT TTTTCTAGTA CCTCAGTCAG CTCCTCTTCC TGTCGGATTG



24701 CTACCACCTT CCCGTCGAG GCACCCCGC TTGAGGAGGA GGAAGTGATT  
 GATGGTGGAA GGGGCAGCTC CGTGGGGGCG AACTCCTCCT CTTTCACTAA  
  
 24751 ATCGAGCAGG ACCCAGGTTT TGTAAGCGAA GACGACGAGG ACCGCTCAGT  
 TAGCTCGTCC TGGGTCCAAA ACATTCGCTT CTGCTGCTCC TGCGGAGTCA  
  
 24801 ACCAACAGAG GATAAAAAGC AAGACCAGGA CAACGCAGAG GCAAACGAGG  
 TGGTTGTCTC CTATTTTTCG TTCTGGTCCT GTTGCGTCTC CGTTTGCTCC  
  
 24851 AACAAAGTCGG GCGGGGGGAC GAAAGGCATG GCGACTACCT AGATGTGGGA  
 TTGTTAGGCC CGCCCCCTG CTTTCCGTAC CGCTGATGGA TCTACACCTT  
  
 24901 GACGACGTGC TGTGAAGCA TCTGCAGCGC CAGTGCGCCA TTATCTGCGA  
 CTGCTGCACG ACAACTTCGT AGACGTCGCG GTCACGCGGT AATAGACGCT  
  
 24951 CGCGTTGCAA GAGCGCAGCG ATGTGCCCCCT CGCCATAGCG GATGTCAGCC  
 GCGCAACGTT CTCGCGTCGC TACACGGGGA GCGGTATCGC CTACAGTCGG  
  
 25001 TTGCCTACGA ACGCCACCTA TTCTCACC GC GTACCCCA CAAACGCCAA  
 AACGGATGCT TCGGGTGGAT AAGAGTGGCG CGCATGGGGG GTTTGCGGTT  
  
 25051 GAAAACGGCA CATGCGAGCC CAACCCGCGC CTCAACTTCT ACCCGGTATT  
 CTTTGGCCGT GTACGCTCGG GTTGGGCGCG GAGTTGAAGA TGGGGCATAA  
  
 25101 TGCCGTGCCA GAGGTGCTTG CCACCTATCA CATCTTTTTC CAAAACCTGCA  
 ACGGCACGGT CTCCACGAAC GGTGGATAGT GTAGAAAAAG GTTTTGACGT  
  
 25151 AGATACCCCT ATCCTGCCGT GCCAACCACA GCCGAGCGGA CAAGCAGCTG  
 TCTATGGGGA TAGGACGGCA CGGTGGCGT CGGCTCGCCT GTTCGTCGAC  
  
 25201 GCCTTGCGGC AGGGCGCTGT CATACTGAT ATCGCCTCGC TCAACGAAGT  
 CGGAACGCCG TCCGCGACA GTATGGACTA TAGCGGAGCG AGTTGCTTCA  
  
 25251 GCCAAAAATC TTTGAGGGTC TTGGACGCGA CGAGAAGCGC GCGGCAACG  
 CGGTTTTTAG AAACCTCCAG AACCTGCGCT GCTCTTCGCG CGCCGTTTGC  
  
 25301 CTCTGCAACA GGAAAACAGC GAAAATGAAA GTCACCTCTG AGTGTTGGTG  
 GAGACGTTGT CTTTTGTGCG CTTTACTTT CAGTGAGACC TCACAACCAC  
  
 25351 GAACTCGAGG GTGACAACGC GCGCCTAGCC GTACTAAAC GCAGCATCGA  
 CTTGAGCTCC CACTGTTGCG CGCGGATCGG CATGATTTTG CGTCGTAGCT  
  
 25401 GGTCACCCAC TTTGCCTACC CGGCACTTAA CCTACCCCCC AAGGTCATGA  
 CCAGTGGGTG AAACGGATGG GCCGTGAATT GGATGGGGGG TTCCAGTACT  
  
 25451 GCACAGTCAT GAGTGAGCTG ATCGTGCGCC GTGCGCAGCC CCTGGAGAGG  
 CGTGTGAGTA CTCACGAC TAGCACGCGG CACGCGTCGG GGACCTCTCC  
  
 25501 GATGCAAATT TGCAAGAACA AACAGAGGAG GGCCTACCCG CAGTTGGCGA  
 CTACGTTTAA ACGTTCTTGT TTGTCTCCTC CCGGATGGGC GTCAACCGCT  
  
 25551 CGAGCAGCTA GCGCGCTGGC TTCAAACGCG CGAGCCTGCC GACTTGGAGG  
 GCTCGTCGAT CGCGCGACCG AAGTTTGCGC GCTCGGACGG CTGAACCTCC

Figure 26 AA

25651 TGCATGCAGC GGTCTTTTGC TGACCCGGAG ATGCAGCGCA AGCTAGAGGA  
ACGTACGTCG CCAAGAAACG ACTGGGCCTC TACGTCGCGT TCGATCTCCT

25701 AACATTGCAC TACACCTTTC GACAGGGCTA CGTACGCCAG GCCTGCAAGA  
TTGTAACGTG ATGTGGAAG CTGTCCCGAT GCATGCGGTC CGGACGTCTT

25751 TCTCCAACGT GGAGCTCTGC AACCTGGTCT CCTACCTTGG AATTTTGAC  
AGAGGTTGCA CCTCGAGACG TTGGACCAGA GGATGGAACC TTAACACGTG

25801 GAAAACCGCC TTGGGCAAAA CGTGCTTCAT TCCACGCTCA AGGGCGAGGC  
CTTTTGGCGG AACCCGTTTT GCACGAAGTA AGGTGCGAGT TCCCGCTCCG

25851 GCGCCGCGAC TACGTCCGCG ACTGCGTTTA CTTATTTCTA TGCTACACCT  
CGCGGCGCTG ATGCAGGCGC TGACGCAAAAT GAATAAAGAT ACGATGTGGA

25901 GGCAGACGGC CATGGGCGTT TGGCAGCAGT GCTTGGAGGA GTGCAACCTC  
CCGTCTGCCG GTACCCGCAA ACCGTCTGTA CGAACCTCCT CACGTTGGAG

25951 AAGGAGCTGC AGAACTGCT AAAGCAAAAC TTGAAGGACC TATGGACGGC  
TTCCTCGACG TCTTTGACGA TTTCGTTTTG AACTTCCTGG ATACCTGCCG

26001 CTTCAACGAG CGCTCCGTGG CCGCGCACCT GGCGGACATC ATTTTCCCCG  
GAAGTTGCTC GCGAGGCACC GGCCTGTGGA CCGCTGTAG TAAAAGGGGC

26051 AACGCCTGCT TAAAACCTG CAACAGGGTC TGCCAGACTT CACCAGTCAA  
TTGCGGACGA ATTTTGGGAC GTTGTCCAG ACGGTCTGAA GTGGTCAGTT

26101 AGCATGTTGC AGAACTTTAG GAACTTTATC CTAGAGCGCT CAGGAATCTT  
TCGTACAACG TCTTGAAATC CTTGAAATAG GATCTCGCGA GTCCTTAGAA

26151 GCCCGCCACC TGCTGTGCAC TTCCTAGCGA CTTTGTGCCC ATTAAGTACC  
CGGGCGGTGG ACGACACGTG AAGGATCGCT GAAACACGGG TAATTCATGG

26201 GCGAATGCCC TCCGCCGCTT TGGGGCCACT GCTACCTTCT GCAGCTAGCC  
CGCTTACGGG AGGCGGCGAA ACCCCGGTGA CGATGGAAGA CGTCGATCGG

26251 AACTACCTTG CCTACCACTC TGACATAATG GAAGACGTGA GCGGTGACGG  
TTGATGGAAC GGATGGTGAG ACTGTATTAC CTTCTGCACT CGCCACTGCC

26301 TCTACTGGAG TGCTACTGTC GCTGCAACCT ATGCACCCCG CACCGCTCCC  
AGATGACCTC ACAGTGACAG CGACGTTGGA TACGTGGGGC GTGGCGAGGG

26351 TGGTTTGCAA TTCGCAGCTG CTTAACGAAA GTCAAATTAT CGGTACCTTT  
ACCAAACGTT AAGCGTCGAC GAATTGCTTT CAGTTTAATA GCCATGGAAA

26401 GAGCTGCAGG GTCCCTCGCC TGACGAAAAG TCCGCGGCTC CGGGGTGAA  
CTCGACGTCC CAGGGAGCGG ACTGCTTTTC AGGCGCCGAG GCCCAACTT

26451 ACTCACTCCG GGGCTGTGGA CGTCGGCTTA CCTTCGCAA TTTGTACCTG  
TGAGTGAGGC CCCGACACCT GCAGCCGAAT GGAAGCGTTT AAACATGGAC

26501 AGGACTACCA CGCCACGAG ATTAGGTTCT ACGAAGACCA ATCCCGCCCC  
TCCTGATGGT GCGGGTGCTC TAATCCAAGA TGCTTCTGGT TAGGGCGGGC

Figure 26 AB

26551 CCTAATGCGG AGCTTACCGC CTGCGTCATT ACCCAGGGCC ACATTCTTGG  
GGATTACGCC TCGAATGGCG GACGCAGTAA TGGGTCCCGG TGTAAGAACC

26601 CCAATTGCAA GCCATCAACA AAGCCCGCCA AGAGTTTCTG CTACGAAAGG  
GGTTAACGTT CGGTAGTTGT TTCGGGCGGT TCTCAAAGAC GATGCTTTCC

26651 GACGGGGGGT TTA CTTGAC CCCCAGTCCG GCGAGGAGCT CAACCCAATC  
CTGCCCCCA AATGAACCTG GGGGTCAGGC CGCTCCTCGA GTTGGGTTAG

26701 CCCCCCGCGC CGCAGCCCTA TCAGCAGCAG CCGCGGGCCC TTGCTTCCCA  
GGGGCGGCG GCGTCGGGAT AGTCGTCGTC GCGCGCCGGG AACGAAGGT

26751 GGATGGCACC CAAAAAGAAG CTGCAGCTGC CGCCGCCACC CACGGACGAG  
CCTACCGTGG GTTTTCTTC GACGTCGACG GCGGCGGTGG GTGCCTGCTC

26801 GAGGAATACT GGGACAGTCA GGCAGAGGAG GTTTTGGACG AGGAGGAGGA  
CTCCTTATGA CCCTGTCAGT CCGTCTCCTC CAAAACCTGC TCCTCCTCCT

26851 GGACATGATG GAAGACTGGG AGAGCCTAGA CGAGGAAGCT TCCGAGGTGC  
CCTGTACTAC CTTCTGACCC TCTCGGATCT GCTCCTTCGA AGGCTCCAGC

26901 AAGAGGTGTC AGACGAAACA CCGTCACCCT CCGTCGCATT CCCCTCGCCG  
TTCTCCACAG TCTGCTTGT GGCAGTGGGA GCCAGCGTAA GGGGAGCGGC

26951 GCGCCCCAGA AATCGGCAAC CGGTTCCAGC ATGGCTACAA CCTCCGCTCC  
CGCGGGGTCT TTAGCCGTTG GCCAAGGTCTG TACCGATGTT GGAGGCGAGG

27001 TCAGGCGCCG CCGGCACTGC CCGTTCGCCG ACCCAACCGT AGATGGGACA  
AGTCCGCGGC GGCCGTGACG GGCAAGCGGC TGGGTTGGCA TCTACCCTGT

27051 CCACTGGAAC CAGGGCCGGT AAGTCCAAGC AGCCGCCGCC GTTAGCCCAA  
GGTGACCTTG GTCCCGGCCA TTCAGGTTCTG TCGGCGGCCG CAATCGGGTT

27101 GAGCAACAAC AGCGCCAAGG CTACCGCTCA TGGCGCGGGC ACAAGAACGC  
CTCGTTGTTG TCGCGGTTCC GATGGCGAGT ACCGCGCCCC TGTTCTTGCG

27151 CATAGTTGCT TGCTTGCAAG ACTGTGGGG CAACATCTCC TTCGCCCGCC  
GTATCAACGA ACGAACGTTT TGACACCCCC GTTGTTAGAGG AAGCGGGCGG

27201 GCTTCTTCT CTACCATCAC GGCGTGGCCT TCCCCGTAA CATCCTGCAT  
CGAAAGAAGA GATGCTAGTG CCGCACC GGA AGGGGGCATT GTAGGACGTA

27251 TACTACCGTC ATCTCTACAG CCCATACTGC ACCGGCGGCA GCGGCAGCAA  
ATGATGGCAG TAGAGATGTC GGGTATGACG TGGCCGCCGT CGCCGTCGTT

27301 CAGCAGCGGC CACACAGAAG CAAAGGCGAC CGGATAGCAA GACTCTGACA  
GTCGTCGCCG GTGTGTCTTC GTTCCGCTG GCCTATCGTT CTGAGACTGT

27351 AAGCCCAAGA AATCCACAGC GCGGCGAGCA GCAGGAGGAG GAGCGCTGCG  
TTCGGGTTCT TTAGGTGTCT CCGCCGTCGT CGTCTCTCTC CTCGCGACGC

27401 TCTGGCGCCC AACGAACCCG TATCGACCCG CGAGCTTAGA AACAGGATTT  
AGACCGCGGG TTGCTTGGGC ATAGCTGGGC GCTCGAATCT TTGTCTTAA

27451 TTCCCACTCT GTATGCTATA TTTCAACAGA GCAGGGGCCA AGAACAAGAG  
AAGGGTGAGA CATACGATAT AAAGTTGTCT CGTCCCCGGT TCTTGTCTC

Figure 26: AC

27551 TCACAAAAGC GAAGATCAGC TTCGGCGCAC GCTGGAAGAC GCGGAGGCTC  
AGTGTTCG CTTCTAGTCG AAGCCGCGTG CGACCTTCTG CGCCTCCGAG

27601 TCTTCAGTAA ATACTGCGCG CTGACTCTTA AGGACTAGTT TCGCGCCCTT  
AGAAGTCATT TATGACGCGC GACTGAGAAT TCCTGATCAA AGCGCGGGAA

27651 TCTCAAATTT AAGCGCGAAA ACTACGTCAT CTCCAGCGGC CACACCCGGC  
AGAGTTTAAA TTCGCGCTTT TGATGCAGTA GAGGTCGCGG GTGTGGGCGG

27701 GCCAGCACCT GTTGTCAGCG CCATTATGAG CAAGGAAATT CCCACGCCCT  
CGGTCGTGGA CAACAGTCGC GGTAACTC GTTCCTTTAA GGGTGCGGGA

27751 ACATGTGGAG TTACCAGCCA CAAATGGGAC TTGCGGCTGG AGCTGCCCAA  
TGTACACCTC AATGGTCGGT GTTACCCTG AACGCCGACC TCGACGGGTT

27801 GACTACTCAA CCCGAATAAA CTACATGAGC GCGGGACCCC ACATGATATC  
CTGATGAGTT GGGCTTATTT GATGTACTCG CGCCCTGGGG TGTACTATAG

27851 CCGGGTCAAC GGAATACGCG CCCACCGAAA CCGAATTCTC CTGGAACAGG  
GGCCCAGTTG CCTTATGCGC GGGTGGCTTT GGCTTAAGAG GACCTTGTC

27901 CGGCTATTAC CACCACACCT CGTAATAACC TTAATCCCCG TAGTTGGCCC  
GCCGATAATG GTGGTGTGGA GCATTATTGG AATTAGGGGC ATCAACCGGG

27951 GCTGCCCTGG TGTACCAGGA AAGTCCCGCT CCCACCACTG TGGTACTTCC  
CGACGGGACC ACATGGTCCT TTCAGGGCGA GGGTGGTGAC ACCATGAAGG

28001 CAGAGACGCC CAGGCCGAAG TTCAGATGAC TAACTCAGGG GCGCAGCTTG  
GTCTCTGCGG GTCCGGCTTC AAGTCTACTG ATTGAGTCCC CGCGTCGAAC

28051 CGGGCGGCTT TCGTCACAGG GTGCGGTCGC CCGGGCAGGG TATAACTCAC  
GCCCCCGAA AGCAGTGTCC CACGCCAGCG GGCCCGTCCC ATATTGAGTG

28101 CTGACAATCA GAGGGCGAGG TATTCAGCTC AACGACGAGT CGGTGAGCTC  
GACTGTTAGT CTCCCGCTCC ATAAGTCGAG TTGCTGCTCA GCCACTCGAG

28151 CTCGCTTGGT CTCCTGCCGG ACGGGACATT TCAGATCGGC GGCGCCGGCC  
GAGCGAACCA GAGGCAGGCC TGCCCTGTAA AGTCTAGCCG CCGCGGCGCG

28201 GCTCTTCATT CACGCCTCGT CAGGCAATCC TAACTCTGCA GACCTCGTCC  
CGAGAAGTAA GTCCGGAGCA GTCCGTAGG ATTGAGACGT CTGGAGCAGG

28251 TCTGAGCCGC GCTCTGGAGG CATTGGAAT CTGCAATTTA TTGAGGAGTT  
AGACTCGGCG CGAGACCTCC GTAACCTTGA GACGTTAAAT AACTCCTCAA

28301 TGTGCCATCG GTCTACTTTA ACCCCTTCTC GGGACCTCCC GGCCACTATC  
ACACGGTAGC CAGATGAAAT TGGGGAAGAG CCCTGGAGGG CCGGTGATAG

28351 CGGATCAATT TATTCCTAAC TTTGACGCGG TAAAGGACTC GGCGGACGGC  
GCCTAGTTAA ATAAGGATTG AAAGTGCGCC ATTTCTTGAG CCGCCTGCGG

28401 TACGACTGAA TGTTAAGTGG AGAGGCAGAG CAACTGCGCC TGAAACACCT  
ATGCTGACTT ACAATTCACC TCTCCGTCTC GTTGACGCGG ACTTTGTGGA

Figure 26 AD

28451 GGTCCACTGT CGCCGCCACA AGTGCTTTGC CCGCGACTCC GGTGAGTTTT  
CCAGGTGACA GCGGCGGTGT TCACGAAACG GCGCGTGAGG CCACTCAAAA

28501 GCTACTTTGA ATTGCCCCGAG GATCATATCG AGGGCCCCGC GCACGGCGTC  
CGATGAAACT TAACGGGCTC CTAGTATAGC TCCCGGGCCG CGTGCCGCGAG

28551 CGGCTTACCG CCCAGGGAGA GCTTGCCCGT AGCCTGATTC GGGAGTTTAC  
GCCGAATGGC GGGTCCCTCT CGAACGGGCA TCGGACTAAG CCCTCAAATG

28601 CCAGCGCCCC CTGCTAGTTG AGCGGGACAG GGGACCCTGT GTTCTCACTG  
GGTCGCGGGG GACGATCAAC TCGCCCTGTC CCCTGGGACA CAAGAGTGAC

28651 TGATTTGCAA CTGTCCTAAC CCTGGATTAC ATCAAGATCT TTGTTGCCAT  
ACTAAACGTT GACAGGATTG GGACCTAATG TAGTTCTAGA AACAACGGTA

28701 CTCTGTGCTG AGTATAATAA ATACAGAAAT TAAATATAC TGGGGCTCCT  
GAGACACGAC TCATATTATT TATGTCTTTA ATTTTATATG ACCCCGAGGA

28751 ATCGCCATCC TGTAACGCC ACCGTCTTCA CCCGCCAAG CAAACCAAGG  
TAGCGGTAGG ACATTGCGG TGGCAGAAGT GGGCGGGTTC GTTTGGTTCC

28801 CGAACCTTAC CTGGTACTTT TAACATCTCT CCCTCTGTGA TTTACAACAG  
GCTTGGAATG GACCATGAAA ATTGTAGAGA GGGAGACACT AAATGTTGTC

28851 TTTCAACCCA GACGGAGTGA GTCTACGAGA GAACCTCTCC GAGCTCAGCT  
AAAGTTGGGT CTGCCTCACT CAGATGCTCT CTTGGAGAGG CTCGAGTCGA

28901 ACTCCATCAG AAAAAACACC ACCCTCCTTA CCTGCCGGGA ACGTACGAGT  
TGAGGTAGTC TTTTTTGTGG TGGGAGGAAT GGACGGCCCT TGCATGCTCA

28951 GCGTCACCGG CCGCTGCACC ACACCTACCG CCTGACCGTA AACCAGACTT  
CGCAGTGGCC GCGGACGTGG TGTGGATGGC GGACTGGCAT TTGGTCTGAA

29001 TTTCCGGACA GACCTCAATA ACTCTGTTTA CCAGAACAGG AGGTGAGCTT  
AAAGGCCTGT CTGGAGTTAT TGAGACAAAT GGTCTTGTC TCCACTCGAA

29051 AGAAAACCCT TAGGGTATTA GGCCAAAGGC GCAGCTACTG TGGGGTTTAT  
TCTTTTGGGA ATCCCATAAT CCGGTTTCCG CGTCGATGAC ACCCCAAATA

29101 GAACAATTCA AGCAACTCTA CGGGCTATTC TAATTCAGGT TTCTCTAGAA  
CTTGTTAAGT TCGTTGAGAT GCCCATAAG ATTAAGTCCA AAGAGATCTT

29151 TCGGGGTTGG GGTATTCTC TGTCTTGTA TTCTCTTTAT TCTTATACTA  
AGCCCCAACC CCAATAAGAG ACAGAACACT AAGAGAAATA AGAATATGAT

29201 ACGCTTCTCT GCCTAAGGCT CGCCGCCTGC TGTGTGCACA TTTGCATTTA  
TGCGAAGAGA CGGATTCCGA GCGGCGGACG ACACACGTGT AAACGTAAAT

29251 TTGTCAGCTT TTAAACGCT GGGGTCGCCA CCCAAGATGA TTAGGTACAT  
AACAGTCGAA AAATTTGCGA CCCAGCGGT GGGTTCTACT AATCCATGTA

29301 AATCCTAGGT TTAATCACCC TTGCGTCAGC CCACGGTACC ACCCAAAGG  
TTAGGATCCA AATGAGTGGG AACGCAGTCG GGTGCCATGG TGGGTTTTCC

29351 TGGATTTTAA GGAGCCAGCC TGTAATGTTA CATTCGCAGC TGAAGCTAAT  
ACCTAAATT CCTCGGTCGG ACATTACAAT GTAAGCGTCG ACTTCGATTA

Figure 26 AE

29451 TCGCCACAAA AACAAAATTG GCAAGTATGC TGTTTATGCT ATTTGGCAGC  
 AGCGGTGTTT TTGTTTAAAC CGTTCATACG ACAAATACGA TAAACCGTCG

29501 CAGGTGACAC TACAGAGTAT AATGTTACAG TTTTCCAGGG TAAAAGTCAT  
 GTCCACTGTG ATGTCTCATA TTACAATGTC AAAAGGTCCC ATTTTCAGTA

29551 AAAACTTTTA TGTATACTTT TCCATTTTAT GAAATGTGCG ACATTACCAT  
 TTTTGAAAAT ACATATGAAA AGGTAAAATA CTTTACACGC TGTAAATGGTA

29601 GTACATGAGC AAACAGTATA AGTTGTGGCC CCCACAAAAT TGTGTGGAAA  
 CATGTACTCG TTTGTCTAT TCAACACCGG GGGTGTTTTA ACACACCTTT

29651 ACACTGGCAC TTTCTGCTGC ACTGCTATGC TAATTACAGT GCTCGCTTTG  
 TGTGACCGTG AAAGACGACG TGACGATACG ATTAATGTCA CGAGCGAAAC

29701 GTCTGTACCC TACTCTATAT TAAATACAAA AGCAGACGCA GCTTTATTGA  
 CAGACATGGG ATGAGATATA ATTTATGTTT TCGTCTGCGT CGAAATAACT

29751 GGAAAAGAAA ATGCCTTAAT TTAATAAGTT ACAAAGCTAA TGTCAACCACT  
 CCTTTTCTTT TACGGAATTA AATGATTCAA TGTTCGATT ACAGTGGTGA

29801 AACTGCTTTA CTCGCTGCTT GCAAAACAAA TTCAAAAAGT TAGCATTATA  
 TTGACGAAAT GAGCGACGAA CGTTTGTGTT AAGTTTTC AATCGTAATAT

29851 ATTAGAATAG GATTTAAACC CCCCGGTCAT TTCCTGCTCA ATACCATTCC  
 TAATCTTATC CTAAATTTGG GGGGCCAGTA AAGGACGAGT TATGGTAAGG

29901 CCTGAACAAT TGACTCTATG TGGGATATGC TCCAGCGCTA CAACCTTGAA  
 GGACTTGTTA ACTGAGATAC ACCCTATACG AGGTCGCGAT GTTGGAACCT

29951 GTCAGGCTTC CTGGATGTCA GCATCTGACT TTGGCCAGCA CCTGTCCCGC  
 CAGTCCGAAG GACCTACAGT CGTAGACTGA AACCGGTCGT GGACAGGGCG

30001 GGATTTGTTT CAGTCCAAC ACAGCGACCC ACCCTAACAG AGATGACCAA  
 CCTAAACAAG GTCAGGTTGA TGTCGCTGGG TGGGATTGTC TCTACTGGTT

30051 CACAACCAAC GCGGCCGCCG CTACCGGACT TACATCTACC ACAAATACAC  
 GTGTTGGTTG CGCCGGCGGC GATGGCCTGA ATGTAGATGG TGTTTATGTG

30101 CCCAAGTTTC TGCTTTTGTC AATAACTGGG ATAACCTGGG CATGTGGTGG  
 GGGTTCAAAG ACGGAAACAG TTATTGACCC TATTGAACCC GTACACCACC

30151 TTCTCCATAG CGCTTATGTT TGTATGCCTT ATTATTATGT GGCTCATCTG  
 AAGAGGTATC GCGAATACAA ACATACGGAA TAATAATACA CCGAGTAGAC

30201 CTGCCTAAAG CGCAAACGCG CCCGACCACC CATCTATAGT CCCATCATTG  
 GACGGATTTT GCGTTTGCGC GGGCTGGTGG GTAGATATCA GGGTAGTAAC

30251 TGCTACACCC AAACAATGAT GGAATCCATA GATTGGACGG ACTGAAACAC  
 ACGATGTGGG TTTGTTACTA CCTTAGGTAT CTAACCTGCC TGACTTTGTG

30301 ATGTTCTTTT CTCTTACAGT ATGATTAAAT GAGACATGAT TCCTCGAGTT  
 TACAAGAAAA GAGAATGTCA TACTAATTTA CTCTGTACTA AGGAGCTCAA

Figure 26 AF

30401 TGCGGTTTCT CACATCGAAG TAGACTGCAT TCCAGCCTTC ACAGTCTATT  
ACGCCAAAAGA GTGTAGCTTC ATCTGACGTA AGGTCGGAAG TGTCAGATAA

30451 TGCTTTACGG ATTTGTCACC CTCACGCTCA TCTGCAGCCT CATCACTGTG  
ACGAAATGCC TAAACAGTGG GAGTGCGAGT AGACGTCGGA GTAGTGACAC

30501 GTCATCGCCT TTATCCAGTG CATTGACTGG GTCTGTGTGC GCTTTGCATA  
CAGTAGCGGA AATAGGTCAC GTAAGTGACC CAGACACACG CGAAACGTAT

30551 TCTCAGACAC CATCCCCAGT ACAGGGACAG GACTATAGCT GAGCTTCTTA  
AGAGTCTGTG GTAGGGGTCA TGTCCCTGTC CTGATATCGA CTCGAAGAAT

30601 GAATTCTTTA ATTATGAAAT TTAGTGTGAC TTTTCTGCTG ATTATTTGCA  
CTTAAGAAAT TAATACTTTA AATGACACTG AAAAGACGAC TAATAAACGT

30651 CCCTATCTGC GTTTTGTTC CCGACCTCCA AGCCTCAAAG ACATATATCA  
GGGATAGACG CAAAACAAGG GGCTGGAGGT TCGGAGTTTC TGTATATAGT

30701 TGCAGATTCA CTCGTATATG GAATATTCCA AGTTGCTACA ATGAAAAAAG  
ACGTCTAAGT GAGCATATAC CTTATAAGGT TCAACGATGT TACTTTTTTC

30751 CGATCTTTCC GAAGCCTGGT TATATGCAAT CATCTCTGTT ATGGTGTCTT  
GCTAGAAAGG CTTGCGACCA ATATACGTTA GTAGAGACAA TACCACAAGA

30801 GCAGTACCAT CTTAGCCCTA GCTATATATC CCTACCTTGA CATTGGCTGG  
CGTCATGGTA GAATCGGGAT CGATATATAG GGATGGAACT GTAACCGACC

30851 AACGCAATAG ATGCCATGAA CCACCCAACT TTCCCCGCGC CCGCTATGCT  
TTGCGTTATC TACGGTACTT GGTGGGTGTA AAGGGGCGCG GCGGATACGA

30901 TCCACTGCAA CAAGTTGTTG CCGGCGGCTT TGTCCCAGCC AATCAGCCTC  
AGGTGACGTT GTTCAACAAC GGCCGCCGAA ACAGGGTCGG TTAGTCGGAG

30951 GCCCACCTTC TCCCACCCCC ACTGAAATCA GCTACTTTAA TCTAACAGGA  
CGGGTGGAAG AGGGTGGGGG TGAATTTAGT CGATGAAATT AGATTGTCTT

31001 GGAGATGACT GACACCCTAG ATCTAGAAAT GGACGGAATT ATTACAGAGC  
CCTCTACTGA CTGTGGGATC TAGATCTTTA CTGCCTTAA TAATGTCTCG

31051 AGCGCCTGCT AGAAAGACGC AGGGCAGCGG CCGAGCAACA GCGCATGAAT  
TCGCGGACGA TCTTTCTGCG TCCCGTCGCC GGCTCGTTGT CCGCTACTTA

31101 CAAGAGCTCC AAGACATGGT TAACTTGAC CAGTGCAAAA GGGGTATCTT  
GTTCTCGAGG TTCTGTACCA ATTGAACGTG GTCACGTTTT CCCCATAGAA

31151 TTGTCTCGTA AAGCAGGCCA AAGTCACCTA CGACAGTAAT ACCACCGGAC  
AACAGAGCAT TTCGTCCGGT TTCAGTGGAT GCTGTCATTA TGGTGGCCTG

31201 ACCGCCTTAG CTACAAGTTG CCAACCAAGC GTCAGAAATT GGTGGTCATG  
TGGCGGAATC GATGTTCAAC GGTGGGTTTC CAGTCTTTAA CCACCAAGTAC

31251 GTGGGAGAAA AGCCCATTA CATAACTCAG CACTCGGTAG AAACCGAAGG  
CACCTCTTT TCGGGTAATG GTATTGAGTC GTGAGCCATC TTTGGCTTCC

Figure 26 AG

31351 AGACCTGTG CGGTCTCAAA GATCTTATTC CCTTTAACTA ATAAAAAAAA  
TCTGGGACAC GCCAGAGTTT CTAGAATAAG GGAAATTGAT TATTTTTTTT

31401 ATAATAAAGC ATCACTTACT TAAAATCAGT TAGCAAATTT CTGTCCAGTT  
TATTATTTCG TAGTGAATGA ATTTTAGTCA ATCGTTTAAA GACAGGTCAA

31451 TATTCAGCAG CACCTCCTTG CCCTCCTCCC AGCTCTGGTA TTGCAGCTTC  
ATAAGTCGTC GTGGAGGAAC GGGAGGAGGG TCGAGACCAT AACGTCGAAG

31501 CTCCTGGCTG CAAACTTTCT CCACAATCTA AATGGAATGT CAGTTTCCTC  
GAGGACCGAC GTTTGAAAGA GGTGTTAGAT TTACCTTACA GTCAAAGGAG

31551 CTGTTCTCTG CCATCCGCAC CCACTATCTT CATGTTGTTG CAGATGAAGC  
GACAAGGACA GGTAGGCGTG GGTGATAGAA GTACAACAAC GTCTACTTCG

31601 GCGCAAGACC GTCTGAAGAT ACCTTCAACC CCGTGTATCC ATATGACACG  
CGCGTTCTGG CAGACTTCTA TGGAAGTTGG GGCACATAGG TATACTGTGC

31651 GAAACCGGTC CTCCAACGTG GCCTTTTCTT ACTCCTCCCT TTGTATCCCC  
CTTTGGCCAG GAGGTTGACA CGGAAAAGAA TGAGGAGGGA AACATAGGGG

31701 CAATGGGTTT CAAGAGAGTC CCCCTGGGGT ACTCTCTTTG CGCCTATCCG  
GTTACCCAAA GTTCTCTCAG GGGGACCCCA TGAGAGAAAC GCGGATAGGC

31751 AACCTCTAGT TACCTCCAAT GGCATGCTTG CGCTCAAAAT GGGCAACGGC  
TTGGAGATCA ATGGAGGTTA CCGTACGAAC GCGAGTTTTA CCCGTTGCCG

31801 CTCTCTCTGG ACGAGGCCGG CAACCTTACC TCCCAAATG TAACCACTGT  
GAGAGAGACC TGCTCCGGCC GTTGGAAATGG AGGGTTTTAC ATTGGTGACA

31851 GAGCCACCT CTCAAAAAA CCAAGTCAAA CATAAACCTG GAAATATCTG  
CTCGGGTGGA GAGTTTTTTT GGTTCAGTTT GTATTTGGAC CTTTATAGAC

31901 CACCCCTCAC AGTTACCTCA GAAGCCCTAA CTGTGGCTGC CGCCGCACCT  
GTGGGGAGTG TCAATGGAGT CTTCCGGGATT GACACCGACG GCGGCGTGGA

31951 CTAATGGTCG CGGGCAACAC ACTCACCATG CAATCACAGG CCCCCTAAC  
GATTACCAGC GCCCGTTGTG TGAGTGGTAC GTTAGTGTCC GGGGCGATTG

32001 CGTGCACGAC TCCAACTTA GCATTGCCAC CCAAGGACCC CTCACAGTGT  
GCACGTGCTG AGGTTTGAAT CGTAACGGTG GGTTCCTGGG GAGTGTCACA

32051 CAGAAGGAAA GCTAGCCCTG CAAACATCAG GCCCCCTCAC CACCACCGAT  
GTCTTCCTTT CGATCGGGAC GTTTGTAGTC CGGGGGAGTG GTGGTGCGTA

32101 AGCAGTACCC TTAATATCAC TGCCTCACC CCTCTAACTA CTGCCACTGG  
TCGTATGCGG AATGATAGTG ACGGAGTGGG GGAGATTGAT GACGGTGACC

32151 TAGCTGGGC ATTGACTTGA AAGAGCCCAT TTATACACAA AATGGAAAAC  
ATCGAACCCG TAACTGAACT TTCTCGGGTA AATATGTGTT TTACCTTTTG

32201 TAGGACTAAA GTACGGGGCT CCTTTGCATG TAACAGACGA CCTAAACACT  
ATCCTGATTT CATGCCCCGA GGAAACGTAC ATTGTCTGCT GGATTTGTGA

Figure 26 AH



32301 AACTAAAGTT ACTGGAGCCT TGGGTTTGA TTCACAAGGC AATATGCAAC  
TTGATTTCAA TGACCTCGGA ACCCAAACT AAGTGTCCG TTATACGTTG

32351 TTAATGTAGC AGGAGGACTA AGGATTGATT CTCAAAACAG ACGCCTTATA  
AATTACATCG TCCTCCTGAT TCCTAACTAA GAGTTTGTG TC CGGAATAT

32401 CTTGATGTTA GTTATCCGTT TGATGCTCAA AACCAACTAA ATCTAAGACT  
GAACTACAAT CAATAGGCAA ACTACGAGTT TTGGTTGATT TAGATTCTGA

32451 AGGACAGGGC CCTCTTTTTA TAAACTCAGC CCACAACCTG GATATTAACT  
TCCTGTCCCG GGAGAAAAAT ATTTGAGTCG GGTGTTGAAC CTATAATTGA

32501 ACAACAAAGG CCTTTACTTG TTTACAGCTT CAAACAATTC CAAAAGCTT  
TGTTGTTTCC GGAAATGAAC AAATGTCGAA GTTTGTAAAG GTTTTTCGAA

32551 GAGGTTAACC TAAGCACTGC CAAGGGGTTG ATGTTTGACG CTACAGCCAT  
CTCCAATTGG ATTCGTGACG GTTCCCAAC TACAACTGC GATGTCGGTA

32601 AGCCATTAAT GCAGGAGATG GGCTTGAATT TGGTTCACCT AATGCACCAA  
TCGGTAATTA CGTCCTCTAC CCGAACTTAA ACCAAGTGGA TTACGTGGTT

32651 ACACAAATCC CCTCAAACA AAAATTGGCC ATGGCCTAGA ATTTGATTCA  
TGTTGTTTAGG GGAGTTTTGT TTTTAACCGG TACCGGATCT TAACTAAGT

32701 AACAAAGGCTA TGGTTCCTAA ACTAGGAAC TGGCCTTAGTT TTGACAGCAC  
TTGTTCCGAT ACCAAGGATT TGATCCTTGA CCGGAATCAA AACTGTCTGT

32751 AGGTGCCATT ACAGTAGGAA AAAAAATAA TGATAAGCTA ACTTTGTGGA  
TCCACGGTAA TGTCATCCTT TGTTTTTATT ACTATTCGAT TGAAACACCT

32801 CCACACCAGC TCCATCTCCT AACTGTAGAC TAAATGCAGA GAAAGATGCT  
GGTGTGGTCG AGGTAGAGGA TTGACATCTG ATTTACGTCT CTTTCTACGA

32851 AAATCACTT TGGTCTTAAC AAAATGTGGC AGTCAAATAC TTGCTACAGT  
TTTGAGTGAA ACCAGAATTG TTTTACACCG TCAGTTTATG AACGATGTCA

32901 TTCAGTTTTG GCTGTAAAG GCAGTTTGGC TCCAATATCT GGAACAGTTC  
AAGTCAAAAC CGACAATTTC CGTCAAACCG AGGTTATAGA CCTGTCAAG

32951 AAAGTGCTCA TCTTATTATA AGATTTGACG AAAATGGAGT GCTACTAAAC  
TTTCACGAGT AGAATAATAT TCTAACTGC TTTTACCTCA CGATGATTTG

33001 AATTCCCTCC TGGACCCAGA ATATTGGAAC TTTAGAAATG GAGATCTTAC  
TTAAGGAAGG ACCTGGGTCT TATAACCTTG AAATCTTTAC CTCTAGAATG

33051 TGAAGGCACA GCCTATACAA ACGCTGTTGG ATTTATGCCT AACCTATCAG  
ACTTCCGTGT CGGATATGTT TGCGACAACC TAAATACGGA TTGGATAGTC

33101 CTTATCCAAA ATCTCACGGT AAAACTGCCA AAAGTAACAT TGTCAGTCAA  
GAATAGGTTT TAGAGTGCCA TTTTGACGGT TTTCATTGTA ACAGTCAGTT

33151 GTTACTTAA ACGGAGACAA AACTAAACCT GTAACACTAA CCATTACACT  
CAAATGAATT TGCCTCTGTT TTGATTTGGA CATTGTGATT GGTAATGTGA

Figure 26 AI

33251 CATTTCATG GGA CTGGTCT GGCCACA ACT ACATTAATGA AATATTTGCC  
GTAAAAGTAC CCTGACCAGA CCGGTGTTGA TGTAAATTACT TTATAAACGG

33301 ACATCCTCTT ACACTTTTTC ATACATTGCC CAAGAATAAA GAATCGTTTG  
TG TAGGAGAA TGTGAAAAAG TATGTAACGG GTTCTTATTT CTTAGCAAA

33351 TGTTATGTTT CAACGTGTTT ATTTTCAAT TGCAGAAAAT TTCAAGTCAT  
ACAATACAAA GTTGACAAA TAAAAAGTTA ACGTCTTTTA AAGTTCAGTA

33401 TTTTCATTCA GTAGTATAGC CCCACCACCA CATAGCTTAT ACAGATCACC  
AAAAGTAAGT CATCATATCG GGGTGGTGGT GTATCGAATA TGTCTAGTGG

33451 GTACCTTAAT CAACTCACA GAACCCTAGT ATTCAACCTG CCACCTCCCT  
CATGGAATTA GTTTGAGTGT CTTGGGATCA TAAGTTGGAC GGTGGAGGGA

33501 CCCAACACAC AGAGTACACA GTCCTTTCTC CCCGGCTGGC CTTAAAAAGC  
GGGTTGTGTG TCTCATGTGT CAGGAAAGAG GGGCCGACCG GAATTTTTCG

33551 ATCATATCAT GGGTAACAGA CATATTCTTA GGTGTTATAT TCCACACGGT  
TAGTATAGTA CCCATTGTCT GTATAAGAAT CCACAATATA AGGTGTGCCA

33601 TTCCTGTGCA GCCAAACGCT CATCAGTGAT ATTAATAAAC TCCCCGGGCA  
AAGGACAGCT CGGTTTGCGA GTAGTCACTA TAATTATTTG AGGGGCCCCGT

33651 GCTCACTTAA GTTCATGTG CTGTCCAGCT GCTGAGCCAC AGGCTGCTGT  
CGAGTGAATT CAAGTACAGC GACAGGTCGA CGACTCGGTG TCCGACGACA

33701 CCAACTTGCG GTTGCTTAAC GGGCGGCGAA GGAGAAGTCC ACGCCTACAT  
GGTTGAACGC CAACGAATTG CCCGCCGCTT CCTCTTCAGG TGCGGATGTA

33751 GGGGGTAGAG TCATAATCGT GCATCAGGAT AGGGCGGTGG TGCTGCAGCA  
CCCCATCTC AGTATTAGCA CGTAGTCCTA TCCCGCCACC ACGACGTCGT

33801 GCGCGCGAAT AAAGTGTGCG CGCCGCCGCT CCGTCCTGCA GGAATACAAC  
CGCGCGCTTA TTTGACGACG GCGGCGGCGA GGCAGGACGT CCTTATGTTG

33851 ATGGCAGTGG TCTCCTCAGC GATGATTGCG ACCGCCCGCA GCATAAGGCG  
TACCGTCACC AGAGGAGTCG CTACTAAGCG TGGCGGGCGT CGTATTCCGC

33901 CCTTGTCCTC CGGGCACAGC AGCGCACCCCT GATCTCACTT AAATCAGCAC  
GGAACAGGAG GCCCGTGTG TCGCGTGGGA CTAGAGTGAA TTTAGTCGTG

33951 AGTAACTGCA GCACAGCACC ACAATATTGT TCAAAATCCC ACAGTGCAAG  
TCATTGACGT CGTGTCGTGG TGTTATAACA AGTTTTAGGG TGTCACGTTT

34001 GCGCTGTATC CAAAGCTCAT GCGGGGGACC ACAGAACCCA CGTGGCCATC  
CGCGACATAG GTTTCGAGTA CCGCCCCTGG TGTCTTGGGT GCACCGGTAG

34051 ATACCACAAG CGCAGGTAGA TTAAGTGGCG ACCCCTCATA AACACGCTGG  
TATGGTGTTC GCGTCCATCT AATTCACCGC TGGGGAGTAT TTGTGCGACC

34101 ACATAAACAT TACCTCTTTT GGCATGTTGT AATTCACCAC CTCCCGGTAC  
TGTATTTGTA ATGGAGAAAA CCGTACAACA TTAAGTGGTG GAGGGCCATG

Figure 26 AJ

34201 GCTGGCCAAA ACCTGCCCCG CGGCTATACA CTGCAGGGAA CCGGGACTGG  
CGACCGGTTT TGGACGGGCG GCCGATATGT GACGTCCCTT GGCCCTGACC

34251 AACAAATGACA GTGGAGAGCC CAGGACTCGT AACCATGGAT CATCATGCTC  
TTGTTACTGT CACCTCTCGG GTCCTGAGCA TTGGTACCTA GTAGTACGAG

34301 GTCATGATAT CAATGTTGGC ACAACACAGG CACACGTGCA TACACTTCCT  
CAGTACTATA GTTACAACCG TGTGTGTCC GTGTGCACGT ATGTGAAGGA

34351 CAGGATTACA AGCTCCTCCC GCGTTAGAAC CATATCCCAG GGAACAACCC  
GTCCTAATGT TCGAGGAGGG CGCAATCTTG GTATAGGGTC CCTTGTGGG

34401 ATTCTTGAAT CAGCGTAAAT CCCACACTGC AGGGAAGACC TCGCACGTAA  
TAAGGACTTA GTCGCATTTA GGGTGTGACG TCCCTTCTGG AGCGTGCATT

34451 CTCACGTTGT GCATTGTCAA AGTGTACAT TCGGGCAGCA GCGGATGATC  
GAGTGCAACA CGTAACAGTT TCACAATGTA AGCCCGTCGT CGCCTACTAG

34501 CTCCAGTATG GTAGCGCGGG TTTCTGTCTC AAAAGGAGGT AGACGATCCC  
GAGGTCATAC CATCGCGCCC AAAGACAGAG TTTTCCTCCA TCTGCTAGGG

34551 TACTGTACGG AGTGCGCCGA GACAACCGAG ATCGTGTGG TCGTAGTGTC  
ATGACATGCC TCACGCGGCT CTGTTGGCTC TAGCACAACC AGCATCACAG

34601 ATGCCAAATG GAACGCCCGA CGTAGTCATA TTTCTTGAAG CAAAACCAGG  
TACGGTTTAC CTTGCGGCCT GCATCAGTAT AAAGGACTTC GTTTTGGTCC

34651 TGCGGGCGTG ACAAACAGAT CTGCGTCTCC GGTCTCGCCG CTTAGATCGC  
ACGCCCGCAC TGTTTGTCTA GACGCAGAGG CCAGAGCGGC GAATCTAGCG

34701 TCTGTGTAGT AGTTGTAGTA TATCCACTCT CTCAAAGCAT CCAGGCGCCC  
AGACACATCA TCAACATCAT ATAGGTGAGA GAGTTTCGTA GGTCCGCGGG

34751 CCTGGCTTCG GGTTCTATGT AAATCCTTC ATGCGCCGCT GCCCTGATAA  
GGACCGAAGC CCAAGATACA TTTGAGGAAG TACGCGGCGA CGGGACTATT

34801 CATCCACCAC CGCAGAATAA GCCACACCCA GCCAACCTAC ACATTCTGTC  
GTAGGTGGTG GCGTCTTATT CGGTGTGGGT CGGTTGGATG TGTAAGCAAG

34851 TGCGAGTCAC ACACGGGAGG AGCGGGAAGA GCTGGAAGAA CCATGTTTTT  
ACGCTCAGTG TGTGCCCTCC TCGCCCTTCT CGACCTTCTT GGTACAAAAA

34901 TTTTTTATTC CAAAAGATTA TCCAAAACCT CAAAATGAAG ATCTATTAAAG  
AAAAAATAAG GTTTTCTAAT AGGTTTGGGA GTTTTACTTC TAGATAATTC

34951 TGAACGCGCT CCCCTCCGGT GCGTGGTCA AACTCTACAG CCAAAGAACA  
ACTTGCGCGA GGGGAGGCCA CCGCACCAGT TTGAGATGTC GGTTTCTTGT

35001 GATAATGGCA TTTGTAAGAT GTTGCACAAT GGCTTCCAAA AGGCAAACGG  
CTATTACCGT AAACATTCTA CAACGTGTTA CCGAAGGTTT TCCGTTTGCC

35051 CCCTCACGTC CAAGTGGACG TAAAGGCTAA ACCCTTCAGG GTGAATCTCC  
GGGAGTGCAG GTTCACCTGC ATTTCCGATT TGGGAAGTCC CACTTAGAGG

Figure 26 AK

35151 CCACCTTCTC AATATATCTC TAAGCAAATC CCGAATATTA AGTCCGGCCA  
GGTGAAGAG TTATATAGAG ATTCGTTTAG GGCTTATAAT TCAGGCCGGT

35201 TTGTAAAAAT CTGCTCCAGA GCGCCCTCCA CCTTCAGCCT CAAGCAGCGA  
AACATTTTTA GACGAGGTCT CGCGGGAGGT GGAAGTCGGA GTTCGTGCGT

35251 ATCATGATTG CAAAAATTCA GGTTCCTCAC AGACCTGTAT AAGATTCAAA  
TAGTACTAAC GTTTTTAAGT CCAAGGAGTG TCTGGACATA TTCTAAGTTT

35301 AGCGGAACAT TAACAAAAAT ACCGCGATCC CGTAGGTCCC TTCGCAGGGC  
TCGCCTTGTA ATTGTTTTTA TGGCGCTAGG GCATCCAGGG AAGCGTCCCG

35351 CAGCTGAACA TAATCGTGCA GGTCTGCACG GACCAGCGCG GCCACTTCCC  
GTGCACTTGT ATTAGCACGT CCAGACGTGC CTGGTCGCGC CGGTGAAGGG

35401 CGCCAGGAAC CATGACAAAA GAACCCACAC TGATTATGAC ACGCATACTC  
GCGGTCCCTG GTACTGTTTT CTTGGGTGTG ACTAATACTG TGCATATGAG

35451 GGAGCTATGC TAACCAGCGT AGCCCCGATG TAAGCTTGTT GCATGGGCGG  
CCTCGATACG ATTGGTCGCA TCGGGGCTAC ATTCGAACAA CGTACCCGCC

35501 CGATATAAAA TGCAAGGTGC TGCTCAAAAA ATCAGGCAAA GCCTCGCGCA  
GCTATATTTT ACGTTCCACG ACGAGTTTTT TAGTCCGTTT CGGAGCGCGT

35551 AAAAAAGAAAG CACATCGTAG TCATGCTCAT GCAGATAAAG GCAGGTAAGC  
TTTTTCTTTC GTGTAGCATC AGTACGAGTA CGTCTATTTC CGTCCATTGC

35601 TCCGGAACCA CCACAGAAAA AGACACCATT TTTCTCTCAA ACATGTCTGC  
AGGCCTTGGT GGTGTCTTTT TCTGTGGTAA AAAGAGAGTT TGTACAGACC

35651 GGGTTTCTGC ATAAACACAA AATAAAATAA CAAAAAACA TTTAAACATT  
CCCAAAGACG TATTTGTGTT TTATTTTATT GTTTTTTTGT AAATTTGTAA

35701 AGAAGCCTGT CTTACAACAG GAAAAACAAC CCTTATAAGC ATAAGACGGA  
TCTTCGGACA GAATGTTGTC CTTTTTGTTG GGAATATTCG TATTCTGCCT

35751 CTACGGCCAT GCCGGCGTGA CCGTAAAAAA ACTGGTCACC GTGATTAAAA  
GATGCCGGTA CGGCCGCACT GGCATTTTTT TGACCAGTGG CACTAATTTT

35801 AGCACCACCG ACAGCTCCTC GGTCAATGTC GGAGTCATAA TGTAAAGACTC  
TCGTGGTGGC TGTCGAGGAG CCAGTACAGG CCTCAGTATT ACATTCTGAG

35851 GGTAAACACA TCAGGTTGAT TCACATCGGT CAGTGCTAAA AAGCGACCGA  
CCATTTGTGT AGTCCAACATA AGTGTAGCCA GTCACGATTT TTCGCTGGCT

35901 AATAGCCCGG GGGAAATACAT ACCCGCAGGC GTAGAGACAA CATTACAGCC  
TTATCGGGCC CCCTTATGTA TGGGCGTCCG CATCTCTGTT GTAATGTCGG

35951 CCCATAGGAG GTATAACAAA ATTAATAGGA GAGAAAAACA CATAAACACC  
GGGTATCCTC CATATTGTTT TAATTATCCT CTCTTTTGT GTATTGTGG

36001 TGAAAAACCC TCCTGCCTAG GCAAAATAGC ACCCTCCCGC TCCAGAACAA  
ACTTTTTGGG AGGACGGATC CGTTTTATCG TGGGAGGGCG AGGTCTTGTT

Figure 26 AL

36101 AAAGAAAACC TATTAACAAA ACACCACTCG ACACGGCACC AGCTCAATCA  
TTTCTTTTGG ATAATTTTTT TGTGGTGAGC TGCGCCGTGG TCGAGTTAGT

36151 GTCACAGTGT AAAAAAGGGC CAAGTGCAGA GCGAGTATAT ATAGGACTAA  
CAGTGTACACA TTTTTTCCCG GTTCACGTCT CGCTCATATA TATCCTGATT

36201 AAAATGACGT AACGGTTAAA GTCCACAAAA AACACCCAGA AAACCGCACG  
TTTACTGCA TTGCCAATTT CAGGTGTTTT TTGTGGGTCT TTTGGCGTGC

36251 CGAACCTACG CCCAGAAACG AAAGCCAAAA AACCCACAAC TTCCTCAAAT  
GCTTGATGC GGGTCTTTGC TTTCGGTTTT TTGGGTGTG AAGGAGTTTA

36301 CGTCACTTCC GTTTTCCCAC GTTACGTCAC TTCCCATTTT AAGAAACTA  
GCAGTGAAGG CAAAAGGGTG CAATGCAGTG AAGGGTAAAA TTCTTTTGAT

36351 CAATTCCCAA CACATACAAG TTAATCCGCC CTAAAACCTA CGTCACCCGC  
GTTAAGGGTT GTGTATGTTT AATGAGGCGG GATTTTGGAT GCAGTGGGCG

36401 CCCGTTCCCA CGCCCCGCGC CACGTCACAA ACTCCACCCC CTCATTATCA  
GGGCAAGGGT GCGGGGCGCG GTGCAGTGTT TGAGGTGGGG GAGTAATAGT

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36451 TATTGGCTTC AATCCAAAAT AAGGTATATT ATTGATGATG TTAATTAAGA  
ATAACCGAAG TTAGGTTTTA TTCCATATAA TAACTACTAC AATTAATTCT

36501 ATTCGGATCT GCGACGCGAG GCTGGATGGC CTCCCCATT ATGATTCTTC  
TAAGCCTAGA CGCTGCGCTC CGACCTACCG GAAGGGGTAA TACTAAGAAG

36551 TCGCTTCCGG CGGCATCGGG ATGCCCCGCT TGCAGGCCAT GCTGTCCAGG  
AGCGAAGGCC GCCGTAGCCC TACGGGCGCA ACGTCCGGTA CGACAGGTCC

36601 CAGGTAGATG ACGACCATCA GGGACAGCTT CAAGGCCAGC AAAAGGCCAG  
GTCCATCTAC TGCTGGTAGT CCCTGTCGAA GTTCCGGTCG TTTTCCGGTC

36651 GAACCGTAAA AAGGCCGCGT TGCTGGCGTT TTCCCATAGG CTCCGCCCCC  
CTTGGCATT TCCGGGCGCA ACGACCGCAA AAAGGTATCC GAGGCGGGGG

36701 CTGACGAGCA TCACAAAAAT CGACGCTCAA GTCAGAGGTG GCGAAACCCG  
GACTGCTCGT AGTGTTTTTA GCTGCGAGTT CAGTCTCCAC CGCTTTGGGC

36751 ACAGGACTAT AAAGATACCA GCGGTTTCCC CCTGGAAGCT CCCTCGTGCG  
TGTCTGATA TTTCTATGGT CCGCAAAGGG GGACCTTCGA GGGAGCACGC

36801 CTCTCTGTG CCGACCCTGC CGCTTACCGG ATACCTGTCC GCCTTTCTCC  
GAGAGGACAA GGCTGGGACG GCGAATGGCC TATGGACAGG CGGAAAGAGG

36851 CTTGCGGAAG CGTGGCGCTT TCTCATAGCT CACGCTGTAG GTATCTCAGT  
GAAGCCCTTC GCACCGCGAA AGAGTATCGA GTGCGACATC CATAGAGTCA

36901 TCGGTGTAGG TCGTTGCTC CAAGCTGGGC TGTGTGCACG AACCCCCCGT  
AGCCACATCC AGCAAGCGAG GTTCGACCCG ACACACGTGC TTGGGGGGCA

Figure 26 AM

37001 CGGTAAGACA CGACTTATCG CCACTGGCAG CAGCCACTGG TAACAGGATT  
GCCATTCTGT GCTGAATAGC GGTGACCGTC GTCGGTGACC ATTGTCCTAA

37051 AGCAGAGCGA GGTATGTAGG CGGTGCTACA GAGTTCTTGA AGTGGTGGCC  
TCGTCTCGCT CCATACATCC GCCACGATGT CTCAAGAACT TCACCACCGG

37101 TAACTACGGC TACACTAGAA GGACAGTATT TGGTATCTGC GCTCTGCTGA  
ATTGATGCCG ATGTGATCTT CCTGTCATAA ACCATAGACG CGAGACGACT

37151 AGCCAGTTAC CTTCGGAAAA AGAGTTGGTA GCTCTTGATC CGGCAAACAA  
TCGGTCAATG GAAGCCTTTT TCTCAACCAT CGAGAACTAG GCCGTTTGT

37201 ACCACCGCTG GTAGCGGTGG TTTTTTTGTT TGCAAGCAGC AGATTACGCG  
TGGTGGCGAC CATCGCCACC AAAAAACAA ACGTTCGTCG TCTAATGCGC

37251 CAGAAAAAAA GGATCTCAAG AAGATCCTTT GATCTTTTCT ACGGGGTCTG  
GTCTTTTTTT CCTAGAGTTC TTCTAGGAAA CTAGAAAAGA TGCCCCAGAC

37301 ACGCTCAGTG GAACGAAAAC TCACGTTAAG GGATTTTGGT CATGAGATTA  
TGCAGATCAC CTTGCTTTTG AGTGCAATTC CCTAAAACCA GTACTCTAAT

37351 TCAAAAAGGA TCTTCACCTA GATCCTTTTA AATCAATCTA AAGTATATAT  
AGTTTTTCCT AGAAGTGGAT CTAGGAAAAT TTAGTTAGAT TTCATATATA

37401 GAGTAACTT GGTCTGACAG TTACCAATGC TTAATCAGTG AGGCACCTAT  
CTCATTTGAA CCAGACTGTC AATGGTTACG AATTAGTCAC TCCGTGGATA

37451 CTCAGCGATC TGTCTATTTT GTTCATCCAT AGTTGCCTGA CTCCCCGTG  
GAGTCGCTAG ACAGATAAAG CAAGTAGGTA TCAACGGACT GAGGGGCAGC

37501 TG TAGATAAC TACGATACGG GAGGGCTTAC CATCTGGCCC CAGTGCTGCA  
ACATCTATTG ATGCTATGCC CTCCCGAATG GTAGACCGGG GTCACGACGT

37551 ATGATACCGC GAGACCCACG CTCACCGGCT CCAGATTTAT CAGCAATAAA  
TACTATGGCG CTCTGGGTGC GAGTGGCCGA GGTCTAAATA GTCGTTATTT

37601 CCAGCCAGCC GGAAGGGCCG AGCGCAGAAG TGGTCCTGCA ACTTTATCCG  
GGTCGGTCGG CCTTCCCGGC TCGCGTCTTC ACCAGGACGT TGAAATAGGC

37651 CCTCCATCCA GTCTATTAAT TGTTGCCGGG AAGCTAGAGT AAGTAGTTCG  
GGAGGTAGGT CAGATAATTA ACAACGGCCC TTCGATCTCA TTCATCAAGC

37701 CCAGTTAATA GTTTGCGCAA CGTTGTTGCC ATTGCTACAG GCATCGTGGT  
GGTCAATTAT CAAACGCGTT GCAACAACGG TAACGATGTC CGTAGCACCA

37751 GTCACGCTCG TCGTTTGGTA TGGCTTCATT CAGCTCCGGT TCCCAACGAT  
CAGTGCGAGC AGCAAACCAT ACCGAAGTAA GTCGAGGCCA AGGGTTGCTA

37801 CAAGGCGAGT TACATGATCC CCCATGTTGT GCAAAAAGC GGTTAGCTCC  
GTTCCGCTCA ATGTACTAGG GGGTACAACA CGTTTTTTCG CCAATCGAGG

37851 TTCGGTCCTC CGATCGTTGT CAGAAGTAAG TTGGCCGCAG TGTATCACT  
AAGCCAGGAG GCTAGCAACA GTCTTCATTC AACCGGCGTC ACAATAGTGA

Figure 26 AN

37951 GATGCTTTTC TGTGACTGGT GAGTACTCAA CCAAGTCATT CTGAGAAATAG  
CTACGAAAAG ACACTGACCA CTCATGAGTT GGTTCAAGTAA GACTCTTATC

38001 TGTATGCGGC GACCGAGTTG CTCTTGCCCG GCGTCAACAC GGGATAATAC  
ACATACGCCG CTGGCTCAAC GAGAACGGGC CGCAGTTGTG CCCTATTATG

38051 CGCGCCACAT AGCAGAACTT TAAAAGTGCT CATCATTGGA AAACGTTCTT  
GCGCGGTGTA TCGTCTTGAA ATTTTCACGA GTAGTAACCT TTTGCAAGAA

38101 CGGGGCGAAA ACTCTCAAGG ATCTTACCGC TGTGAGATC CAGTTCGATG  
GCCCCGCTTT TGAGAGTTCC TAGAATGGCG ACAACTCTAG GTCAAGCTAC

38151 TAACCCACTC GTGCACCCAA CTGATCTTCA GCATCTTTTA CTTTCACCAG  
ATTGGGTGAG CACGTGGGTT GACTAGAAGT CGTAGAAAAT GAAAGTGGTC

38201 CGTTTCTGGG TGAGCAAAAA CAGGAAGGCA AAATGCCGCA AAAAAGGGAA  
GCAAAGACCC ACTCGTTTTT GTCCCTCCGT TTTACGGCGT TTTTCCCTT

38251 TAAGGGCGAC ACGGAAATGT TGAATACTCA TACTCTTCCT TTTTCAATAT  
ATTCCCGCTG TGCCTTTACA ACTTATGAGT ATGAGAAGGA AAAAGTTATA

38301 TATTGAAGCA TTTATCAGGG TTATTGTCTC ATGAGCGGAT ACATATTTGA  
ATAACTTCGT AAATAGTCCC AATAACAGAG TACTCGCCTA TGTATAAACT

38351 ATGTATTTAG AAAAATAAAC AAATAGGGGT TCCGCGCACA TTTCCCCGAA  
TACATAAATC TTTTATTTG TTTATCCCCA AGGCGCGTGT AAAGGGGCTT

38401 AAGTGCCACC TGACGTCTAA GAAACCATTA TTATCATGAC ATTAACCTAT  
TTCACGGTGG ACTGCAGATT CTTTGGAAT AATAGTACTG TAATTGGATA

38451 AAAAATAGGC GTATCACGAG GCCCTTTCGT CTTCAAGAAT TGGATCCGAA  
TTTTTATCCG CATAGTGCTC CGGGAAAGCA GAAGTTCTTA ACCTAGGCTT

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38501 TTCTTAATTT CTTAATTAA (SEQ ID NO:32)  
AAGAATTAAA GAATTAATT (SEQ ID NO:33)

Figure 26 A0

1 CATCATCAAT AATATACCTT ATTTTGGATT GAAGCCAATA TGATAATGAG  
GTAGTAGTTA TTATATGGAA TAAAACCTAA CTTCGGTTAT ACTATTACTC

51 GGGGTGGAGT TTGTGACGTG GCGCGGGGCG TGGGAACGGG GCGGGTGACG  
CCCCACCTCA AACACTGCAC CGCGCCCCGC ACCCTTGCCC CGCCCACTGC

101 TAGTAGTGTG GCGGAAAGTG GATGTTGCAA GTGTGGCGGA ACACATGTAA  
ATCATCACAC CGCCTTCACA CTACAACGTT CACACCGCCT TGTGTACATT

151 GCGACGGATG TGGCAAAAGT GACGTTTTTG GTGTGCGCCG GTGTACACAG  
CGCTGCCTAC ACCGTTTTCA CTGCAAAAAC CACACGCGGC CACATGTGTC

201 GAAGTGACAA TTTTCGCGCG GTTTTAGGCG GATGTTGTAG TAAATTTGGG  
CTTCACTGTT AAAAGCGCGC CAAATCCGC CTACAACATC ATTTAAACCC

251 CGTAACCGAG TAAGATTTGG CCATTTTCGC GGGAAACTG AATAAGAGGA  
GCATTGGCTC ATTCTAAACC GGTAAAAGCG CCCTTTTGAC TTATTCTCCT

301 AGTGAAATCT GAATAATTTT GTGTTACTCA TAGCGCGTAA TATTTGTCTA  
TCACCTTAGA CTTATTAAAA CACAATGAGT ATCGCGCATT ATAAACAGAT

351 GGGCGCGGG GACTTTGACC GTTTACGTGG AGACTCGCCC AGGTGTTTTT  
CCCCGCGCCC CTGAACTGG CAAATGCACC TCTGAGCGGG TCCACAAAAA

401 CTCAGGTGTT TTCCGCGTTC CGGGTCAAAG TTGGCGTTTT ATTATTATAG  
GAGTCCACAA AAGGCGCAAG GCCCAGTTTC AACCGCAAAA TAATAATATC

451 GCGGCCGCGA TCCATTGCAT ACGTTGTATC CATATCATAA TATGTACATT  
CGCCGGCGCT AGGTAACGTA TGCAACATAG GTATAGTATT ATACATGTAA

501 TATATTGGCT CATGTCCAAC ATTACCGCCA TGTGACATT GATTATTGAC  
ATATAACCGA GTACAGGTTG TAATGGCGGT ACAACTGTAA CTAATAACTG

551 TAGTTATTAA TAGTAATCAA TTACGGGGTC ATTAGTTCAT AGCCCATATA  
ATCAATAATT ATCATTAGTT AATGCCCCAG TAATCAAGTA TCGGGTATAT

601 TGGAGTTCCG CGTTACATAA CTTACGGTAA ATGGCCCGCC TGGCTGACCG  
ACCTCAAGGC GCAATGTATT GAATGCCATT TACCGGGCGG ACCGACTGGC

651 CCCAACGACC CCCGCCATT GACGTCAATA ATGACGTATG TTCCCATAGT  
GGGTTGCTGG GGGCGGGTAA CTGCAGTTAT TACTGCATAC AAGGGTATCA

701 AACGCCAATA GGGACTTTCC ATTGACGTCA ATGGGTGGAG TATTTACGGT  
TTGCGGTTAT CCCTGAAAGG TAACTGCAGT TACCCACCTC ATAAATGCCA

751 AAAGTCCCCA CTTGGCAGTA CATCAAGTGT ATCATATGCC AAGTACGCCC  
TTTGACGGGT GAACCGTCAT GTAGTTCACA TAGTATACGG TTCATGCGGG

801 CCTATTGACG TCAATGACGG TAAATGGCCC GCCTGGCATT ATGCCCAGTA  
GGATAACTGC AGTTACTGCC ATTTACCGGG CGGACCGTAA TACGGGTCAAT

Figure 27A



901 TCGCTATTAC CATGGTGATG CGGTTTTGGC AGTACATCAA TGGGCGTGGA  
AGCGATAATG GTACCACTAC GCCAAAACCG TCATGTAGTT ACCCGCACCT

951 TAGCGGTTTG ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA  
ATCGCCAAAC TGAGTGCCCC TAAAGGTTCA GAGGTGGGGT AACTGCAGTT

1001 TGGGAGTTTG TTTTGGCACC AAAATCAACG GGACTTTCCA AAATGTCGTA  
ACCCCTCAAAC AAAACCGTGG TTTTAGTTGC CCTGAAAGGT TTTACAGCAT

1051 ACAACTCCGC CCCATTGACG CAAATGGGCG GTAGGCGTGT ACGGTGGGAG  
TGTTGAGGCG GGGTAACTGC GTTTACCCGC CATCCGCACA TGCCACCCTC

1101 GTCTATATAA GCAGAGCTCG TTTAGTGAAC CGTCAGATCG CCTGGAGACG  
CAGATATATT CGTCTCGAGC AAATCACTTG GCAGTCTAGC GGACCTCTGC

1151 CCATCCACGC TGTMTTGACC TCCATAGAAG ACACCGGGAC CGATCCAGCC  
GGTAGGTGCG ACAAACCTGG AGGTATCTTC TGTGGCCCTG GCTAGGTGCG

1201 TCCGCGGCCG GGAACGGTGC ATTGGAACGC GGATTCCCCG TGCCAAGAGT  
AGGCGCCCGC CCTTGCCACG TAACCTTGCG CTAAGGGGC ACGGTTCTCA

1251 GAGATCTGCC ACCATGGCCG GCAAGTGGTC CAAGAGGTCC GTGCCCGGCT  
CTCTAGACGG TGGTACCGGC CGTTCACCAG GTTCTCCAGG CACGGGCCGA

1301 GGTCCACCGT GAGGGAGAGG ATGAGGAGGG CCGAGCCCGC CGCCGACAGG  
CCAGGTGGCA CTCCCTCTCC TACTCCTCCC GGCTCGGGCG GCGGCTGTCC

1351 GTGAGGAGGA CCGAGCCCGC CGCAGTGGGC GTGGGCGCCG TGTCCAGGGA  
CACTCCTCCT GGCTCGGGCG GCGTCACCCG CACCCGCGGC ACAGGTCCCT

1401 CCTGGAGAAG CACGGCGCCA TCACCTCCTC CAACACCGCC GCCACCAACG  
GGACCTCTTC GTGCCGCGGT AGTGGAGGAG GTTGTGGCGG CGGTGGTTGC

1451 CCGACTGCGC CTGGCTGGAG GCCCAGGAGG ACGAGGAGGT GGGCTTCCCC  
GGCTGACGCG GACCGACCTC CGGGTCCTCC TGCTCCTCCA CCCGAAGGGG

1501 GTGAGGCCCC AGGTGCCCCCT GAGGCCCATG ACCTACAAGG GCGCCGTGGA  
CACTCCGGGG TCCACGGGGA CTCCGGGTAC TGATGTTCC CGCGGCACCT

1551 CCTGTCCAC TTCCTGAAGG AGAAGGGCGG CCTGGAGGGC CTGATCCACT  
GGACAGGGTG AAGGACTTCC TCTTCCCGCC GGACCTCCCG GACTAGGTGA

1601 CCCAGAAGAG GCAGGACATC CTGGACCTGT GGGTGTACCA CACCCAGGGC  
GGGTCTTCTC CGTCCTGTAG GACCTGGACA CCCACATGGT GTGGGTCCCG

1651 TACTTCCCCG ACTGGCAGAA CTACACCCCC GGCCCCGGCA TCAGGTTCCC  
ATGAAGGGGC TGACCGTCTT GATGTGGGGG CCGGGGCCGT AGTCCAAGGG

1701 CCTGACCTTC GGCTGGTGCT TCAAGCTGGT GCCCGTGGAG CCCGAGAAGG  
GGACTGGAAG CCGACCACGA AGTTCGACCA CGGGCACCTC GGGCTCTTCC

1751 TGGAGGAGGC CAACGAGGGC GAGAACAAC TCGCCGCCCA CCCCATGTCC  
ACCTCCTCCG GTTGCTCCCG CTCTTGTTGA CGCGGCGGGT GGGGTACAGG

Figure 27B

1851 CTCCAAGCTG GCCTTCCACC ACGTGGCCAG GGAGCTGCAC CCCGAGTACT  
GAGGTTTCGAC CGGAAGGTGG TGCACCGGTC CCTCGACGTG GGGCTCATGA

1901 ACAAGGACTG CTAAAGCCCG GGCAGATCTG CTGTGCCTTC TAGTTGCCAG  
TGTTTCCTGAC GATTTCGGGC CCGTCTAGAC GACACGGAAG ATCAACGGTC

1951 CCATCTGTTG TTTGCCCCCTC CCCCCTGCCT TCCTTGACCC TGGAAGGTGC  
GGTAGACAAC AAACGGGGAG GGGGCACGGA AGGAACTGGG ACCTTCCACG

2001 CACTCCCCTG GTCCTTTTCT AATAAAATGA GGAAATTGCA TCGCATTGTC  
GTGAGGGTGA CAGGAAAGGA TTATTTTACT CCTTTAACGT AGCGTAACAG

2051 TGAGTAGGTG TCATTCTATT CTGGGGGGTG GGTGGGGCA GGACAGCAAG  
ACTCATCCAC AGTAAGATAA GACCCCCAC CCCACCCCGT CCTGTCGTTT

2101 GGGGAGGATT GGAAGACAA TAGCAGGCAT GCTGGGGATG CGGTGGGCTC  
CCCCTCCTAA CCCTTCTGTT ATCGTCCGTA CGACCCCTAC GCCACCCGAG

2151 TATGGCCGAT CGGCGCGCCG TACTGAAATG TGTGGGCGTG GCTTAAGGGT  
ATACCGGCTA GCCGCGCGGC ATGACTTTAC ACACCCGCAC CGAATTCCCA

2201 GGGAAAGAAT ATATAAGGTG GGGGTCTTAT GTAGTTTGT ATCTGTTTTG  
CCCTTTCTTA TATATTCCAC CCCCAGAATA CATCAAAACA TAGACAAAC

2251 CAGCAGCCGC CGCCGCCATG AGCACCAACT CGTTTGATGG AAGCATTGTG  
GTCGTCGGCG GCGGCGGTAC TCGTGTTGA GCAAACTACC TTCGTAACAC

2301 AGCTCATATT TGACAACGCG CATGCCCCCA TGGGCCGGGG TGCCTCAGAA  
TCGAGTATAA ACTGTTGCGC GTACGGGGGT ACCCGGCCCC ACGCAGTCTT

2351 TGTGATGGGC TCCAGCATTG ATGGTCGCCC CGTCTGCCC GCAAACTCTA  
ACACTACCCG AGGTCGTAAC TACCAGCGGG GCAGGACGGG CGTTTGAGAT

2401 CTACCTTGAC CTACGAGACC GTGTCTGGAA CGCCGTTGGA GACTGCAGCC  
GATGGAAC TGATGCTCTGG CACAGACCTT GCGGCAACCT CTGACGTCGG

2451 TCCGCCGCCG CTTAGCCGC TGCAGCCACC GCGCGGGGA TTGTGACTGA  
AGGCGGCGGC GAAGTCGGCG ACGTCGGTGG CGGGCGCCCT AACACTGACT

2501 CTTTGCTTTC CTGAGCCCGC TTGCAAACAG TGCAGCTTCC CGTTCATCCG  
GAAACGAAAG GACTCGGGCG AACGTTTGTC ACGTCGAAGG GCAAGTAGGC

2551 CCCGCGATGA CAAGTTGACG GCTCTTTTGG CACAATTGGA TTCTTTGACC  
GGGCGCTACT GTTCAACTGC CGAGAAAACC GTGTTAACCT AAGAACTGG

2601 CGGGAACCTA ATGTCGTTTC TCAGCAGCTG TTGGATCTGC GCCAGCAGGT  
GCCCTTGAAT TACAGCAAAG AGTCGTCGAC AACCTAGACG CGGTCGTCCA

2651 TTCTGCCCTG AAGGCTTCCT CCCCTCCCAA TGCGGTTTAA AACATAAATA  
AAGACGGGAC TTCCGAAGGA GGGGAGGGTT ACGCCAAATT TTGTATTTAT

2701 AAAAACCAGA CTCTGTTTGG ATTTGGATCA AGCAAGTGTC TTGCTGTCTT  
TTTTTGGTCT GAGACAAACC TAAACCTAGT TCGTTCACAG AACGACAGAA

Figure 27C

2751 TATTTAGGGG TTTTGCGCGC GCGGTAGGCC CGGGACCAGC GGTCTCGGTC  
ATAAATCCCC AAAACGCGCG CGCCATCCGG GCCCTGGTCG CCAGAGCCAG

2801 GTTGAGGGTC CTGTGTATTT TTTCCAGGAC GTGGTAAAGG TGACTCTGGA  
CAACTCCCAG GACACATAAA AAAGGTCCTG CACCATTTC ACTGAGACCT

2851 TGTTCAGATA CATGGGCATA AGCCCGTCTC TGGGGTGGAG GTAGCACCAC  
ACAAGTCTAT GTACCCGTAT TCGGGCAGAG ACCCCACCTC CATCGTGGTG

2901 TGCAGAGCTT CATGCTGCGG GGTGGTGTG TAGATGATCC AGTCGTAGCA  
ACGTCTCGAA GTACGACGCC CCACCACAAC ATCTACTAGG TCAGCATCGT

2951 GGAGCGCTGG GCGTGGTGCC TAAAAATGTC TTTTCAGTAGC AAGCTGATTG  
CCTCGCGACC CGCACCACGG ATTTTACAG AAAGTCATCG TTCGACTAAC

3001 CCAGGGGCAG GCCCTTGGTG TAAGTGTTTA CAAAGCGGTT AAGCTGGGAT  
GGTCCCCGTC CGGGAACCAC ATTCACAAAT GTTCGCCAA TTCGACCCTA

3051 GGGTGCATAC GTGGGGATAT GAGATGCATC TTGGACTGTA TTTTATAGTT  
CCCACGTATG CACCCCTATA CTCTACGTAG AACCTGACAT AAAATCCAA

3101 GGCTATGTTT CCAGCCATAT CCCTCCGGGG ATTCATGTTG TGCAGAACCA  
CCGATACAAG GGTCCGTATA GGGAGGCCCC TAAGTACAAC ACGTCTTGGT

3151 CCAGCACAGT GTATCCGGTG CACTTGGGAA ATTTGTATG TAGCTTAGAA  
GGTCGTGTCA CATAGGCCAC GTGAACCCTT TAAACAGTAC ATCGAATCTT

3201 GGAAATGCGT GGAAGAACTT GGAGACGCCC TTGTGACCTC CAAGATTTTC  
CCTTTACGCA CTTCTTGAA CCTCTGCGGG AACACTGGAG GTTCTAAAAG

3251 CATGCATTCG TCCATAATGA TGGCAATGGG CCCACGGGCG GCGGCCTGGG  
GTACGTAAGC AGGTATTACT ACCGTTACCC GGGTGCCCCG CGCCGAGCCC

3301 CGAAGATATT TCTGGGATCA CTAACGTCAT AGTTGTGTTT CAGGATGAGA  
GCTTCTATAA AGACCCTAGT GATTGCAGTA TCAACACAAG GTCCTACTCT

3351 TCGTCATAGG CCATTTTAC AAAGCGCGG CGGAGGGTGC CAGACTGCGG  
AGCAGTATCC GGTAAAAATG TTTCGCGCCC GCCTCCACG GTCTGACGCC

3401 TATAATGGTT CCATCCGGCC CAGGGGCGTA GTTACCCTCA CAGATTTGCA  
ATATTACCAA GGTAGGCCGG GTCCCCGCAT CAATGGGAGT GTCTAAACGT

3451 TTTCCACGC TTTGAGTTCA GATGGGGGGA TCATGTCTAC CTGCGGGGCG  
AAAGGGTGCG AAACCTCAAGT CTACCCCTT AGTACAGATG GACGCCCCGC

3501 ATGAAGAAA CGGTTTCCGG GGTAGGGGAG ATCAGCTGGG AAGAAAGCAG  
TACTTCTTTT GCCAAAGGCC CCATCCCTC TAGTCGACCC TTCTTTCGTC

3551 GTTCCTGAGC AGCTGCGACT TACCGCAGCC GGTGGGCCCC TAAATCACAC  
CAAGGACTCG TCGACGCTGA ATGGCGTCGG CCACCCGGGC ATTTAGTGTG

3601 CTATTACCGG CTGCAACTGG TAGTTAAGAG AGCTGCAGCT GCCGTATCC  
GATAATGGCC GACGTTGACC ATCAATTCTC TCGACGTCGA CGGCAGTAGG

3651 CTGAGCAGGG GGGCCACTTC GTTAAGCATG TCCCTGACTC GCATGTTTTT  
GACTCGTCCC CCCGGTGAAG CAATTCTGAC AGGGACTGAG CGTACAAAAG

3701 CCTGACCAAA TCCGCCAGAA GGCGCTCGCC GCCCAGCGAT AGCAGTTCTT  
 GGACTGGTTT AGGCGGTCTT CCGCGAGCGG CGGGTCGCTA TCGTCAAGAA  
  
 3751 GCAAGGAAGC AAAGTTTTTC AACGGTTTGA GACCGTCCGC CGTAGGCATG  
 CGTTCCTTCG TTTCAAAAAG TTGCCAAACT CTGGCAGGCG GCATCCGTAC  
  
 3801 CTTTTGAGCG TTTGACCAAG CAGTTCCAGG CGGTCCCACA GCTCGGTCAC  
 GAAAACTCGC AAACGTGGTC GTCAAGGTCC GCCAGGGTGT CGAGCCAGTG  
  
 3851 CTGCTCTACG GCATCTCGAT CCAGCATATC TCCTCGTTTC GCGGGTTGGG  
 GACGAGATGC CGTAGAGCTA GGTCTGTATAG AGGAGCAAAG CGCCCAACCC  
  
 3901 GCGGCTTTTCG CTGTACGGCA GTAGTCGGTG CTCGTCCAGA CGGGCCAGGG  
 CGCCGAAAGC GACATGCCGT CATCAGCCAC GAGCAGGTCT GCCCGGTCCC  
  
 3951 TCATGTCTTT CCACGGGCGC AGGGTCCTCG TCAGCGTAGT CTGGGTCACG  
 AGTACAGAAA GGTGCCCCGCG TCCCAGGAGC AGTCGCATCA GACCCAGTGC  
  
 4001 GTGAAGGGGT GCGCTCCGGG CTGCGCGCTG GCCAGGGTGC GCTTGAGGCT  
 CACTTCCCCA CGCGAGGCCC GACGCGCGAC CGGTCCCACG CGAACTCCGA  
  
 4051 GGTCTTGCTG GTGCTGAAGC GCTGCCGGTC TTCGCCCTGC GCGTCGGCCA  
 CCAGGACGAC CACGACTTCG CGACGGCCAG AAGCGGGACG CGCAGCCGCT  
  
 4101 GGTCAGATTT GACCATGGTG TCATAGTCCA GCCCCTCCGC GGCGTGCCCC  
 CCATCGTAAA CTGGTACCAC AGTATCAGGT CGGGGAGGCG CCGCACCGGG  
  
 4151 TTGGCGCGCA GCTTGCCCTT GGAGGAGGCG CCGCACGAGG GGCAGTGCAG  
 AACCGCGCGT CGAACGGGAA CCTCCTCCGC GGCGTGCTCC CCGTCACGTC  
  
 4201 ACTTTTGAGG GCGTAGAGCT TGGGCGCGAG AAATACCGAT TCCGGGGAGT  
 TGAAACTCC CGCATCTCGA ACCCGCGCTC TTTATGGCTA AGGCCCCCTCA  
  
 4251 AGGCATCCGC GCCGCAGGCC CCGCAGACGG TCTCGCATTC CACGAGCCAG  
 TCCGTAGGCG CGGCGTCCGG GCGTCTGCCC AGAGCGTAAG GTGCTCGGTC  
  
 4301 GTGAGCTCTG GCCGTTCCGG GTCAAAAACC AGGTTTCCCC CATGCTTTTT  
 CACTCGAGAC CGGCAAGCCC CAGTTTTTGG TCCAAAGGGG GTACGAAAAA  
  
 4351 GATGCGTTTC TTACCTCTGG TTTCCATGAG CCGGTGTCCA CGCTCGGTGA  
 CTACGCAAAG AATGGAGACC AAAGGTACTC GGCCACAGGT GCGAGCCACT  
  
 4401 CGAAAAGGCT GTCCGTGTCC CCGTATACAG ACTTGAGAGG CCTGTCTCTG  
 GCTTTTCCGA CAGGCACAGG GGCATATGTC TGAACCTCTC GGACAGGAGC  
  
 4451 AGCGGTGTTC CGCGGTCTCT CTCGTATAGA AACTCGGACC ACTCTGAGAC  
 TCGCCACAAG GCGCCAGGAG GAGCATATCT TTGAGCCTGG TGAGACTCTG  
  
 4501 AAAGGCTCGC GTCCAGGCCA GCACGAAGGA GGCTAAGTGG GAGGGGTAGC  
 TTTCCGAGCG CAGGTCCGGT CGTGCTTCCT CCGATTACAC CTCCCCATCG  
  
 4551 GGTGCTTGTC CACTAGGGGG TCCACTCGCT CCAGGGTGTG AAGACACATG  
 CCAGCAACAG GTGATCCCCC AGGTGAGCGA GGTCCCACAC TTCTGTGTAC  
  
 4601 TCGCCCTCTT CGGCATCAAG GAAGGTGATT GGTGTGTAGG TGTAGGCCAC  
 AGCGGGAGAA GCCGTAGTTC CTTCCACTAA CCAAACATCC ACATCCGGTG

Figure 27E

4701 CGTCCTCACT CTCTTCCGCA TCGCTGTCTG CGAGGGCCAG CTGTTGGGGT  
GCAGGAGTGA GAGAAGGCGT AGCGACAGAC GCTCCCGGTC GACAACCCCA

4751 GAGTACTCCC TCTGAAAAGC GGGCATGACT TCTGCGCTAA GATTGTCAGT  
CTCATGAGGG AGACTTTTCG CCCGTACTGA AGACGCGATT CTAACAGTCA

4801 TTCCAAAAAC GAGGAGGATT TGATATTCAC CTGGCCCGCG GTGATGCCTT  
AAGGTTTTTG CTCTCCTAA ACTATAAGTG GACCGGGCGC CACTACGGAA

4851 TGAGGGTGGC CGCATCCATC TGGTCAGAAA AGACAATCTT TTTGTTGTCA  
ACTCCACCG GCGTAGGTAG ACCAGTCTTT TCTGTTAGAA AAACAACAGT

4901 AGCTTGGTGG CAAACGACCC GTAGAGGGCG TTGGACAGCA ACTTGGCGAT  
TCGAACCACC GTTTGCTGGG CATCTCCCGC AACCTGTCGT TGAACCGCTA

4951 GGAGCGCAGG GTTTGGTTTT TGTCGCGATC GCGCGCTCC TTGGCCGCGA  
CCTCGCGTCC CAAACCAAAA ACAGCGCTAG CCGCGCGAGG AACCGGCGCT

5001 TGTTTAGCTG CACGTATTCG CGCGCAACGC ACCGCCATTC GGGAAAGACG  
ACAAATCGAC GTGCATAAGC GCGCGTTGCG TGGCGGTAAG CCCTTTCTGC

5051 GTGGTGCCT CGTCGGGCAC CAGGTGCACG CGCCAACCGC GGTGTGTCAG  
CACCACGCGA GCAGCCCGTG GTCCACGTGC GCGGTTGGCG CCAACACGTC

5101 GGTGACAAGG TCAACGCTGG TGGCTACCTC TCCGCGTAGG CGCTCGTTGG  
CCACTGTTCC AGTTGCGACC ACCGATGGAG AGGCGCATCC GCGAGCAACC

5151 TCCAGCAGAG GCGGCCGCCC TTGCGCGAGC AGAATGGCGG TAGGGGGTCT  
AGGTGCTCTC CGCCGGCGGG AACGCGCTCG TCTTACCGCC ATCCCCCAGA

5201 AGCTGCGTCT CGTCCGGGGG GTCTGCGTCC ACGGTAAAGA CCCCGGGCAG  
TCGACGCAGA GCAGGCCCCC CAGACGCAGG TGCCATTTCT GGGGCCCGTC

5251 CAGGCGCGCG TCGAAGTAGT CTATCTTGCA TCCTTGCAAG TCTAGCGCCT  
GTCCGCGCGC AGCTTCATCA GATAGAACGT AGGAACGTTT AGATCGCGGA

5301 GCTGCCATGC GCGGGCGGCA AGCGCGCGCT CGTATGGGTT GAGTGGGGGA  
CGACGGTACG CGCCCGCCGT TCGCGCGCGA GCATACCCAA CTCACCCCT

5351 CCCCATGGCA TGGGGTGGGT GAGCGCGGAG GCGTACATGC CGCAAATGTC  
GGGGTACCGT ACCCCACCCA CTCGCGCCTC CGCATGTACG GCGTTTACAG

5401 GTAAACGTAG AGGGGCTCTC TGAGTATTCC AAGATATGTA GGGTAGCATC  
CATTTGCATC TCCCCGAGAG ACTCATAAGG TTCTATACAT CCCATCGTAG

5451 TTCCACCGCG GATGCTGGCG CGCACGTAAT CGTATAGTTC GTGCGAGGGA  
AAGGTGGCGC CTACGACCGC GCGTGCATTA GCATATCAAG CACGCTCCCT

5501 GCGAGGAGGT CGGGACCGAG GTTGCTACGG GCGGGCTGCT CTGCTCGGAA  
CGCTCCTCCA GCCCTGGCTC CAACGATGCC CGCCCGACGA GACGAGCCTT

5551 GACTATCTGC CTGAAGATGG CATGTGAGTT GGATGATATG GTTGACGCT  
CTGATAGACG GACTTCTACC GTACACTCAA CCTACTATAC CAACCTGCGA

Figure 27F

5651 GAGGCGTAGG AGTCGCGCAG CTTGTTGACC AGCTCGGCGG TGACCTGCAC  
CTCCGCATCC TCAGCGCGTC GAACAACTGG TCGAGCCGCC ACTGGACGTG

5701 GTCTAGGGCG CAGTAGTCCA GGGTTTCCTT GATGATGTCA TACTTATCCT  
CAGATCCCGC GTCATCAGGT CCCAAAGGAA CTACTACAGT ATGAATAGGA

5751 GTCCCTTTTT TTTCCACAGC TCGCGGTTGA GGACAACTC TTCGCGGTCT  
CAGGGAAAAA AAAGGTGTCT AGCGCCAACT CCTGTTTGAG AAGCGCCAGA

5801 TTCCAGTACT CTTGGATCGG AAACCCGTCG GCCTCCGAAC GGTAAGAGCC  
AAGGTCATGA GAACCTAGCC TTTGGGCAGC CGGAGGCTTG CCATTCTCGG

5851 TAGCATGTAG AACTGGTTGA CGGCCTGGTA GGCGCAGCAT CCCTTTTCTA  
ATCGTACATC TTGACCAACT GCCGGACCAT CCGCGTCGTA GGGAAAAGAT

5901 CGGGTAGCGC GTATGCCTGC GCGGCCTTCC GGAGCGAGGT GTGGGTGAGC  
GCCCATCGCG CATACGGACG CGCCGGAAGG CCTCGCTCCA CACCCACTCG

5951 GCAAAGGTGT CCCTGACCAT GACTTTGAGG TACTGGTATT TGAAGTCAGT  
CGTTTCCACA GGGACTGGTA CTGAACTCC ATGACCATAA ACTTCAGTCA

6001 GTCGTCGCAT CCGCCCTGCT CCCAGAGCAA AAAGTCCGTG CGCTTTTTGG  
CAGCAGCGTA GCGGGGACGA GGGTCTCGTT TTTCAGGCAC GCGAAAAACC

6051 AACGCGGATT TGGCAGGGCG AAGGTGACAT CGTTGAAGAG TATCTTTCCC  
TTGCGCCTAA ACCGTCCCGC TTCCACTGTA GCAACTTCTC ATAGAAAGGG

6101 GCGCGAGGCA TAAAGTTGCG TGTGATGCGG AAGGGTCCCG GCACCTCGGA  
CGCGTCCGT ATTTCAACGC AACTACGCC TTCCAGGGC CGTGGAGCCT

6151 ACGGTTGTTA ATTACCTGGG CGGCGAGCAC GATCTCGTCA AAGCCGTTGA  
TGCCAACAAT TAATGGACCC GCCGCTCGTG CTAGAGCAGT TTCGGCAACT

6201 TGTGTGGGCC CACAATGTAA AGTTCCAAGA AGCGCGGGAT GCCCTTGATG  
ACAACACCGG GTGTTACATT TCAAGGTTCT TCGCGCCCTA CGGGAACACT

6251 GAAGGCAATT TTTTAAGTTC CTCGTAGGTG AGCTCTTCAG GGGAGCTGAG  
CTTLCGTTAA AAAATTCAAG GAGCATCCAC TCGAGAAGTC CCCTCGACTC

6301 CCCGTGCTCT GAAAGGGCCC AGTCTGCAAG ATGAGGGTTG GAAGCGACGA  
GGGCACGAGA CTTTCCCGGG TCAGACGTTT TACTCCCAAC CTTGCTGCT

6351 ATGAGCTCCA CAGGTCACGG GCCATTAGCA TTTGCAGGTG GTCGCGAAAG  
TACTCGAGGT GTCCAGTGCC CGGTAATCGT AAACGTCCAC CAGCGCTTTC

6401 GTCCTAAACT GGCGACCTAT GGCCATTTTT TCTGGGGTGA TGCAGTAGAA  
CAGGATTTGA CCGCTGGATA CCGGTAAAAA AGACCCCACT ACGTCATCTT

6451 GGTAAGCGGG TCTTGTTCCC AGCGGTCCCA TCCAAGGTTT GCGGCTAGGT  
CCATTGCCCC AGAACAAGGG TCGCCAGGGT AGGTTCCAAG CGCCGATCCA

6501 CTCGCGCGGC AGTCACTAGA GGCTCATCTC CGCCGAACTT CATGACCAGC  
GAGCGCGCCG TCAGTGATCT CCGAGTAGAG GCGGCTTGAA GTACTGGTCTG

Figure 27G

6601 TACATCGTAG GTGACAAAGA GACGCTCGGT GCGAGGATGC GAGCCGATCG  
 ATGTAGCATC CACTGTTTCT CTGCGAGCCA CGCTCCTACG CTCGGCTAGC  
 6651 GGAAGAACTG GATCTCCCGC CACCAATTGG AGGAGTGGCT ATTGATGTGG  
 CCTTCTTGAC CTAGAGGGCG GTGGTTAACC TCCTCACCGA TAACTACACC  
 6701 TGAAAGTAGA AGTCCCTGCG ACGGGCCGAA CACTCGTGCT GGCTTTTGTA  
 ACTTTCATCT TCAGGGACGC TGCCCGGCTT GTGAGCACGA CCGAAAACAT  
 6751 AAAACGTGCG CAGTACTGGC AGCGGTGCAC GGGCTGTACA TCCTGCACGA  
 TTTTGCACGC GTCATGACCG TCGCCACGTG CCCGACATGT AGGACGTGCT  
 6801 GGTTGACCTG ACGACCGCGC ACAAGGAAGC AGAGTGGGAA TTTGAGCCCC  
 CCAACTGGAC TGCTGGCGCG GTTTCCTTCG TCTACCCCTT AACTCGGGG  
 6851 TCGCCTGGCG GGTTTGGCTG GTGGTCTTCT ACTTCGGCTG CTTGTCCTTG  
 AGCGGACCGC CCAAACCGAC CACCAGAAGA TGAAGCCGAC GAACAGGAAC  
 6901 ACCGTCTGGC TGCTCGAGGG GAGTTACGGT GGATCGGACC ACCACGCCGC  
 TGGCAGACCG ACGAGCTCCC CTCAATGCCA CCTAGCCTCG TGGTGGCGCG  
 6951 GCGAGCCCAA AGTCCAGATG TCCGCGCGCG GCGGTGCGAG CTTGATGACA  
 CGCTCGGGTT TCAGGTCTAC AGGCGCGCGC CGCCAGCCTC GAACTACTGT  
 7001 ACATCGCGCA GATGGGAGCT GTCCATGGTC TGGAGCTCCC GCGGCGTCAG  
 TGTAGCGCGT CTACCCTCGA CAGGTACCAG ACCTCGAGGG CGCCGCAGTC  
 7051 GTCAGGCGGG AGCTCCTGCA GGTTTACCTC GCATAGACGG GTCAGGGCGC  
 CAGTCCGCC CCGAGGACGT CCAAATGGAG CGTATCTGCC CAGTCCCGCG  
 7101 GGGCTAGATC CAGGTGATAC CTAATTTCCA GGGGCTGGTT GGTGGCGGCG  
 CCCGATCTAG GTCCACTATG GATTAAAGGT CCCCACCAA CCACCGCCGC  
 7151 TCGATGGCTT GCAAGAGGCC GCATCCCCGC GCGCGACTA CGGTACCGCG  
 AGCTACCGAA CGTTCTCCGG CGTAGGGGCG CCGCGCTGAT GCCATGGCGC  
 7201 CGGCGGGCGG TGGGCCGCGG GGGTGTCTT GGATGATGCA TCTAAAAGCG  
 GCCGCCCGCC ACCCGGCGCC CCCACAGGAA CCTACTACGT AGATTTTCGC  
 7251 GTGACGCGGG CGAGCCCCCG GAGGTAGGGG GGGCTCCGGA CCCGCCGGGA  
 CACTGCGCCC GCTCGGGGGC CTCCATCCCC CCCGAGGCCT GGGCGGCCCT  
 7301 GAGGGGGCAG GGGCACGTCG GCGCCGCGCG CGGGCAGGAG CTGGTGCTGC  
 CTCCCCGTC CCCGTGCAGC CGCGGCGCGC GCCCGTCTC GACCACGACG  
 7351 GCGCGTAGGT TGCTGGCGAA CGCGACGACG CGGCGGTGA TCTCCTGAAT  
 CGCGCATCCA ACGACCGCTT GCGCTGCTGC GCCGCCAACT AGAGGACTTA  
 7401 CTGGCGCCTC TGCGTGAAGA CGACGGGCCC GGTGAGCTTG AACCTGAAAG  
 GACCGCGGAG ACGCACTTCT GCTGCCCCGG CCACCTCGAAC TTGGACTTTC  
 7451 AGAGTTCGAC AGAATCAATT TCGGTGTCGT TGACGGCGGC CTGGCGCAA  
 TCTCAAGCTG TCTTAGTTAA AGCCACAGCA ACTGCCGCCG GACCGCGTTT

Figure 27H

7551 CTGCTCGATC TCTTCCTCCT GGAGATCTCC GCGTCCGGCT CGCTCCACGG  
GACGAGCTAG AGAAGGAGGA CCTCTAGAGG CGCAGGCCGA GCGAGGTGCG

7601 TGGCGGCGAG GTCGTTGGAA ATGCGGGCCA TGAGCTGCGA GAAGGCGTTG  
ACCGCCGCTC CAGCAACCTT TACGCCCCTG ACTCGACGCT CTTCCGCAAC

7651 AGGCCTCCCT CGTTCAGAC GCGGCTGTAG ACCACGCCCC CTTCGGCATC  
TCCGAGGGA GCAAGGTCTG CGCCGACATC TGGTGCGGGG GAAGCCGTAG

7701 GCGGGCGCGC ATGACCACCT GCGCGAGATT GAGCTCCACG TGCCGGGCGA  
CGCCCGCGCG TACTGGTGA CGCGCTCTAA CTCGAGGTGC ACGGCCCGCT

7751 AGACGGCGTA GTTTCGAGG CGCTGAAAGA GGTAGTTGAG GGTGGTGGCG  
TCTGCCGCAT CAAAGCGTCC GCGACTTTCT CCATCAACTC CCACCACCGC

7801 GTGTGTTCTG CCACGAAGAA GTACATAACC CAGCGTCGCA ACGTGGATTG  
CACACAAGAC GGTGCTTCTT CATGTATTGG GTCGCAGCGT TGCACCTAAG

7851 GTTGATATCC CCCAAGGCCT CAAGGCGCTC CATGGCCTCG TAGAAGTCCA  
CAACTATAGG GGGTCCGGA GTTCCGCGAG GTACCGGAGC ATCTTCAGGT

7901 CGGCGAAGTT GAAAACTGG GAGTTGCGCG CCGACACGGT TAACTCCTCC  
GCCGCTTCAA CTTTTTGACC CTCAACGCGC GGCTGTGCCA ATTGAGGAGG

7951 TCCAGAAGAC GGATGAGCTC GCGGACAGTG TCGCGCACCT CGCGCTCAA  
AGGTCTTCTG CCTACTCGAG CCGCTGTAC AGCGCGTGA GCGCGAGTTT

8001 GGCTACAGGG GCCTCTTCTT CTTCTTCAAT CTCCTCTTCC ATAAGGGCCT  
CCGATGTCCC CGGAGAAGAA GAAGAAGTTA GAGGAGAAGG TATTCCTCGA

8051 CCCCTTCTTC TTCTTCTGGC GCGGGTGGGG GAGGGGGGAC ACGCGGGCGA  
GGGAAGAAG AAGAAGACCG CCGCCACCCC CTCCCCCTG TGCCCGCGCT

8101 CGACGGCGCA CCGGGAGGCG GTCGACAAAG CGCTCGATCA TCTCCCCGCG  
GCTGCCGCGT GGCCCTCCGC CAGCTGTTTC GCGAGCTAGT AGAGGGGCGC

8151 GCGACGGCGC ATGGTCTCGG TGACGGCGCG GCCGTTCTCG CGGGGGCGCA  
CGCTGCCGCG TACCAGAGCC ACTGCCGCGC CGGCAAGAGC GCGCCCGCGT

8201 GTTGGAAGAC GCCGCCCCGTC ATGTCCCGGT TATGGGTTGG CGGGGGGCTG  
CAACCTTCTG CGGCGGGCAG TACAGGGCCA ATACCAACC GCGCCCGGAC

8251 CCATGCGGCA GGGATACGGC GCTAACGATG CATCTCAACA ATTGTTGTGT  
GGTACGCCGT CCCTATGCCG CGATTGCTAC GTAGAGTTGT TAACAACACA

8301 AGGTACTCCG CCGCCGAGGG ACCTGAGCGA GTCCGCATCG ACCGGATCGG  
TCCATGAGGC GCGGGCTCCC TGGACTCGCT CAGGCGTAGC TGGCCTAGCC

8351 AAAACCTCTC GAGAAAGGCG TCTAACCAGT CACAGTCGCA AGGTAGGCTG  
TTTTGGAGAG CTCTTTCCGC AGATTGGTCA GTGTCAGCGT TCCATCCGAC

8401 AGCACCGTGG CGGGCGGCG CCGGCGGCG TCGGGGTTGT TTCTGGCGGA  
TCGTGGCACC GCGCGCCGTC GCGCGCCGCC AGCCCAACA AAGACCGCCT

Figure 27I



8501 TCGACAGAAG CACCATGTCC TTGGGTCCGG CCTGCTGAAT GCGCAGGCGG  
AGCTGTCTTC GTGGTACAGG AACCCAGGCC GGACGACTTA CGCGTCCGCC

8551 TCGGCCATGC CCCAGGCTTC GTTTTGACAT CGGCGCAGGT CTTTGTAGTA  
AGCCGGTACG GGGTCCGAAG CAAAACGTGA GCCGCGTCCA GAAACATCAT

8601 GTCTTGCAATG AGCCTTTCTA CCGGCACTTC TTCTTCTCCT TCCTCTTGTC  
CAGAACGTAC TCGGAAAGAT GGCCGTGAAG AAGAAGAGGA AGGAGAACAG

8651 CTGCATCTCT TGCATCTATC GCTGCGGCGG CGGCGGAGTT TGGCCGTAGG  
GACGTAGAGA ACGTAGATAG CGACGCCGCC GCCGCCTCAA ACCGGCATCC

8701 TGGCGCCCTC TTCCTCCCAT GCGTGTGACC CCGAAGCCCC TCATCGGCTG  
ACCGCGGGAG AAGGAGGGTA CGCACACTGG GGCTTCGGGG AGTAGCCGAC

8751 AAGCAGGGCT AGGTCGGCGA CAACGCGCTC GGCTAATATG GCCTGCTGCA  
TTCGTCCCGA TCCAGCCGCT GTTGC GCGAG CCGATTATAC CGGACGACGT

8801 CCTGCGTGAG GGTAGACTGG AAGTCATCCA TGTCCACAAA GCGGTGGTAT  
GGACGCACTC CCATCTGACC TTCAGTAGGT ACAGGTGTTT CGCCACCATA

8851 GCGCCCGTGT TGATGGTGTG AGTGCAGTTG GCCATAACGG ACCAGTTAAC  
CGCGGGCACA ACTACCACAT TCACGTCAAC CGGTATTGCC TGGTCAATTG

8901 GGTCTGGTGA CCCGGCTGCG AGAGCTCGGT GTACCTGAGA CGCGAGTAAG  
CCAGACCACT GGGCCGACGC TCTCGAGCCA CATGGACTCT GCGCTCATTC

8951 CCCTCGAGTC AAATACGTAG TCGTTGCAAG TCCGCACCAG GTACTGGTAT  
GGGAGCTCAG TTTATGCATC AGCAACGTTT AGGCGTGGTC CATGACCATA

9001 CCCACCAAAA AGTGCGGCGG CGGCTGGCGG TAGAGGGGCC AGCGTAGGGT  
GGGTGGTTTT TCACGCCGCC GCCGACCGCC ATCTCCCCGG TCGCATCCCA

9051 GGCCGGGGCT CCGGGGGCGA GATCTTCCAA CATAAGGCGA TGATATCCGT  
CCGGCCCCGA GGCCCCGCT CTAGAAGGTT GTATTCCGCT ACTATAGGCA

9101 AGATGTACCT GGACATCCAG GTGATGCCGG CGGCGGTGGT GGAGGCGCGC  
TCTACATGGA CCTGTAGGTC CACTACGCC GCCGCCACCA CCTCCGCGCG

9151 GGAAAGTCGC GGACGCGGTT CCAGATGTTG CGCAGCGGCA AAAAGTGCTC  
CCTTTCAGCG CCTGCGCCAA GGTCTACAAC GCGTCGCCGT TTTTCACGAG

9201 CATGGTCGGG ACGCTCTGGC CGGTACGGCG CGCGCAATCG TTGACGCTCT  
GTACCAGCCC TGCAGAGCCG GCCAGTCCGC GCGCGTTAGC AACTGCGAGA

9251 AGACCGTGCA AAAGGAGAGC CTGTAAGCGG GCACTCTTCC GTGGTCTGGT  
TCTGGCACGT TTTCTCTCG GACATTCGCC CGTGAGAAGG CACCAGACCA

9301 GGATAAATTC GCAAGGGTAT CATGGCGGAC GACCGGGGTT CGAGCCCCGT  
CCTATTTAAG CGTTCCATA GTACCGCCTG CTGGCCCCAA GCTCGGGGCA

9351 ATCCGGCCGT CCGCCGTGAT CCATGCGGTT ACCGCCCGCG TGTCGAACCC  
TAGGCCGGCA GGCGGCACTA GGTACGCCAA TGGCGGGCGC ACAGCTTGGG

Figure 27J

9451 GGC GCG GCG CG CTGCTGCGCT AGCTTTTTTG GCCACTGGCC GCGCGCAGCG  
 CCGCGCCGCC GACGACGCGA TCGAAAAAAC CCGTGACCGG CCGCGCTCGC  
 9501 TAAGCGGTTA GGCTGGAAAG CGAAAGCATT AAGTGGCTCG CTCCTGTAG  
 ATTCGCCAAT CCGACCTTTC GCTTTCGTAA TTCACCGAGC GAGGGACATC  
 9551 CCGGAGGGTT ATTTTCCAAG GGTGAGTCG CGGGACCCCC GGTTCGAGTC  
 GGCCTCCCAA TAAAAGGTTT CCAACTCAGC GCCCTGGGGG CCAAGCTCAG  
 9601 TCGGACCGGC CGGACTGCGG CGAACGGGGG TTTGCCTCCC CGTCATGCAA  
 AGCCTGGCCG GCCTGACGCC GCTTGCCCCC AAACGGAGGG GCAGTACGTT  
 9651 GACCCCGCTT GCAAATTCCT CCGGAAACAG GGACGAGCCC CTTTTTTGCT  
 CTGGGGCGAA CGTTTAAGGA GGCCTTTGTC CCTGCTCGGG GAAAAAACGA  
 9701 TTTCCAGAT GCATCCGGTG CTGCGGCAGA TGCGCCCCC TCCTCAGCAG  
 AAAGGGTCTA CGTAGGCCAC GACGCCGTCT ACGCGGGGGG AGGAGTCGTC  
 9751 CGGCAAGAGC AAGAGCAGCG GCAGACATGC AGGGCACCCCT CCCCTCCTCC  
 GCCGTTCTCG TTCTCGTCGC CGTCTGTACG TCCCGTGGGA GGGGAGGAGG  
 9801 TACCGCGTCA GGAGGGGCGA CATCCGCGGT TGACGCGGCA GCAGATGGTG  
 ATGGCGCAGT CCTCCCCGCT GTAGGCGCCA ACTGCGCCGT CGTCTACCAC  
 9851 ATTACGAACC CCCGCGGCGC CGGGCCCCGC ACTACCTGGA CTTGGAGGAG  
 TAATGCTTGG GGGCGCCGCG GCCCGGGCCG TGATGGACCT GAACCTCCTC  
 9901 GCGGAGGGCC TGGCGCGGCT AGGAGCGCCC TCTCCTGAGC GGCACCCAAG  
 CCGCTCCCGG ACCGCGCCGA TCCTCGCGGG AGAGGACTCG CCGTGGGTTT  
 9951 GGTGCAGCTG AAGCGTGATA CGCGTGAGGC GTACGTGCCG CGGCAGAACC  
 CCACGTCGAC TTCGCACTAT GCGCACTCCG CATGCACGGC GCCGTCTTGG  
 10001 TGTTTCGCGA CCGCGAGGGA GAGGAGCCCG AGGAGATGCG GGATCGAAAG  
 ACAAAGCGCT GCGGCTCCCT CTCCTCGGGC TCCTCTACGC CCTAGCTTTC  
 10051 TTCCACGCAG GCGCGAGCT GCGGCATGGC CTGAATCGCG AGCGGTTGCT  
 AAGGTGCGTC CCGCGCTCGA CGCCGTACCG GACTTAGCGC TCGCCAACGA  
 10101 GCGCGAGGAG GACTTTGAGC CCGACGCGCG AACCAGGATT AGTCCCAGCG  
 CGCGCTCCTC CTGAAACTCG GGCTGCGCGC TTGGCCCTAA TCAGGGCGCG  
 10151 GCGCACACGT GCGGCGCGCC GACCTGGTAA CCGCATACGA GCAGACGGTG  
 CGCGTGTGCA CCGCGGCGCG CTGGACCATT GCGGTATGCT CGTCTGCCAC  
 10201 AACCAGGAGA TTAACCTTCA AAAAAAGCTTT AACAAACCAG TGCGTACGCT  
 TTGGTCTCTT AATTGAAAGT TTTTTCGAAA TTGTTGGTGC ACGCATGCGA  
 10251 TGTGGCGCGC GAGGAGGTGG CTATAGGACT GATGCATCTG TGGGACTTTG  
 ACACCGCGCG CTCCTCCACC GATATCCTGA CTACGTAGAC ACCCTGAAAC  
 10301 TAAGCGCGCT GGAGCAAAAC CCAAATAGCA AGCCGCTCAT GCGCGAGCTG  
 ATTCGCGCGA CTCGTTTTG GGTATTATCGT TCGGCGAGTA CCGCGTCGAC

Figure 27K

10401 GCTAAACATA GTAGAGCCCG AGGGCCGCTG GCTGCTCGAT TTGATAAACA  
 CGATTTGTAT CATCTCGGGC TCCCGGCGAC CGACGAGCTA AACTATTTGT  
 10451 TCCTGCAGAG CATAGTGGTG CAGGAGCGCA GCTTGAGCCT GGCTGACAAG  
 AGGACGTCTC GTATCACCAC GTCCTCGCGT CGAACTCGGA CCGACTGTTC  
 10501 GTGGCCGCCA TCAACTATTC CATGCTTAGC CTGGGCAAGT TTTACGCCCCG  
 CACCGGCGGT AGTTGATAAG GTACGAATCG GACCCGTTCA AAATGCGGGC  
 10551 CAAGATATAC CATACCCCCTT ACGTTCCCAT AGACAAGGAG GTAAAGATCG  
 GTTCTATATG GTATGGGGAA TGCAAGGGTA TCTGTTCCTC CATTTCTAGC  
 10601 AGGGGTTCTA CATGCGCATG GCGCTGAAGG TGCTTACCTT GAGCGACGAC  
 TCCCCAAGAT GTACGCGTAC CGCGACTTCC ACGAATGGAA CTCGCTGCTG  
 10651 CTGGGCGTTT ATCGCAACGA GCGCATCCAC AAGGCCGTGA GCGTGAGCCG  
 GACCCGCAA TAGCGTTGCT CGCGTAGGTG TTCCGGCACT CGCACTCGGC  
 10701 GCGGCGCGAG CTCAGCGACC GCGAGCTGAT GCACAGCCTG CAAAGGGCCC  
 CGCCGCGCTC GAGTCGCTGG CGCTCGACTA CGTGTCGGAC GTTTCCTGGG  
 10751 TGGCTGGCAC GGGCAGCGGC GATAGAGAGG CCGAGTCCTA CTTTGACGCG  
 ACCGACCGTG CCCGTCGCCG CTATCTCTCC GGCTCAGGAT GAAACTGCGC  
 10801 GCGGCTGACC TGGCTGGGC CCCAAGCCGA CGCGCCCTGG AGGCAGCTGG  
 CCGCGACTGG ACGCGACCCG GGGTTCGGCT GCGCGGGACC TCCGTCGACC  
 10851 GGCCGGACCT GGGCTGGCGG TGGCACCCGC GCGCGCTGGC AACGTGCGCG  
 CCGGCCTGGA CCCGACCGCC ACCGTGGGCG CGCGCGACCG TTGCAGCCGC  
 10901 GCGTGGAGGA ATATGACGAG GACGATGAGT ACGAGCCAGA GGACGGCGAG  
 CGCACCTCCT TATACTGCTC CTGCTACTCA TGCTCGGTCT CTGCGCGCTC  
 10951 TACTAAGCGG TGATGTTTCT GATCAGATGA TGCAAGACGC AACGGACCCG  
 ATGATTGCGC ACTACAAAGA CTAGTCTACT ACGTTCTGCG TTGCCTGGGC  
 11001 GCGGTGCGGG CGGCGCTGCA GAGCCAGCCG TCCGGCCTTA ACTCCACGGA  
 CGCCACGCC CCCGCGACGT CTCGGTCGGC AGGCCGGAAT TGAGGTGCCT  
 11051 CGACTGGCGC CAGGTCATGG ACCGCATCAT GTCGCTGACT GCGCGCAATC  
 GCTGACCGCG GTCCAGTACC TGGCGTAGTA CAGCGACTGA CGCGCGTTAG  
 11101 CTGACGCGTT CCGGCAGCAG CCGCAGGCCA ACCGGCTCTC CGCAATTCTG  
 GACTGCGCAA GGCCGTCGTC GCGTCCGGT TGGCCGAGAG GCGTTAAGAC  
 11151 GAAGCGGTGG TCCCGGCGCG CGCAAACCCC ACGCACGAGA AGGTGCTGGC  
 CTTGCGCCACC AGGGCCGCGC GCGTTTGGGG TGCGTGCTCT TCCACGACCG  
 11201 GATCGTAAAC GCGCTGGCCG AAAACAGGGC CATCCGGCCC GACGAGGCCG  
 CTAGCATTTG CGCGACCGGC TTTGTCCCG GTAGGCCGGG CTGCTCCGGC  
 11251 GCCTGGTCTA CGACGCGCTG CTTAGCGCG TGGCTCGTTA CAACAGCGGC  
 CGGACCAGAT GCTGCGCGAC GAAGTCGCGC ACCGAGCAAT GTTGTGCGCG

Figure 27L

11351	GGCGCAGCGT	GAGCGCGCGC	AGCAGCAGGG	CAACCTGGGC	TCCATGGTTG
	CCGCGTCGCA	C7CGCGCGC	TCGTCGTCCC	GTTGGACCCG	AGGTACCAAC
11401	CACTAAACGC	CTTCCTGAGT	ACACAGCCCG	CCAACGTGCC	GCGGGGACAG
	GTGATTTGCG	GAAGGACTCA	TGTGTCGGGC	GTTTGCACGG	CGCCCTGTCT
11451	GAGGACTACA	CCAACCTTGT	GAGCGCACTG	CGGCTAATGG	TGACTGAGAC
	CTCCTGATGT	GGTTGAAACA	CTCGCGTGAC	GCCGATTACC	ACTGACTCTG
11501	ACCGCAAAGT	GAGGTGTACC	AGTCTGGGCC	AGACTATTTT	TTCCAGACCA
	TGGCGTTTCA	CTCCACATGG	TCAGACCCGG	TCTGATAAAA	AAGGTCTGGT
11551	GTAGACAAGG	CCTGCAGACC	GTAAACCTGA	GCCAGGCTTT	CAAAAACCTG
	CATCTGTTCC	GGACGTCTGG	CATTGTGGACT	CGGTCCGAAA	GTTTTTGAAC
11601	CAGGGGCTGT	GGGGGGTGCG	GGCTCCACAC	GGCGACCGCG	CGACCGTGTC
	GTCCCCGACA	CCCCCACGCG	CCGAGGGTGT	CCGCTGGCGC	GCTGGCACAG
11651	TAGCTTGCTG	ACGCCCAACT	CGCGCCTGTT	GCTGCTGCTA	ATAGCGCCCT
	ATCGAACGAC	TGCGGGTTGA	GCGCGGACAA	CGACGACGAT	TATCGCGGGA
11701	TCACGGACAG	TGGCAGCGTG	TCCCGGGACA	CATACCTAGG	TCACTTGCTG
	AGTGCCTGTC	ACCGTCGCAC	AGGGCCCTGT	GTATGGATCC	AGTGAACGAC
11751	ACACTGTACC	GCGAGGCCAT	AGGTCAGGCG	CATGTGGACG	AGCATACTTT
	TGTGACATGG	CGCTCCGGTA	TCCAGTCCGC	GTACACCTGC	TCGTATGAAA
11801	CCAGGAGATT	ACAAGTGTC	GCCGCGCGCT	GGGGCAGGAG	GACACGGGCA
	GGTCTCTTAA	TGTTACACAGT	CGGCGCGCGA	CCCCGTCTCT	CTGTGCCCGT
11851	GCCTGGAGGC	AACCCTAAAC	TACCTGCTGA	CCAACCGGCG	GCAGAAGATC
	CGGACCTCCG	TTGGGATTTG	ATGGACGACT	GGTTGGCCGC	CGTCTTCTAG
11901	CCCTCGTTGC	ACAGTTTAAA	CAGCGAGGAG	GAGCGCATTT	TGCGCTACGT
	GGGAGCAACG	TGTCAAATTT	GTCGCTCCTC	CTCGCGTAAA	ACGCGATGCA
11951	GCAGCAGAGC	GTGAGCCTTA	ACCTGATGCG	CGACGGGGTA	ACGCCCAGCG
	CGTCGTCTCG	CACCTCGGAAT	TGGACTACGC	GCTGCCCCAT	TGCGGGTTCG
12001	TGGCGCTGGA	CATGACCGCG	CGCAACATGG	AACCGGGCAT	GTATGCCTCA
	ACCGCGACCT	GTA CTGGCGC	GCGTTGTACC	TTGGCCCGTA	CATACGGAGT
12051	AACCGGCCGT	TTATCAACCG	CCTAATGGAC	TACTTGATC	GCGCGGCCGC
	TTGGCCGGCA	AATAGTTGGC	GGATTACCTG	ATGAACGTAG	CGCGCCGGCG
12101	CGTGAACCCC	GAGTATTTCA	CCAATGCCAT	CTTGAACCCG	CACTGGCTAC
	GCACTTGGGG	CTCATAAAGT	GGTTACGGTA	GAACTTGGGC	GTGACCGATG
12151	CGCCCCCTGG	TTTCTACACC	GGGGGATTTC	AGGTGCCCCG	GGGTAACGAT
	GCGGGGGACC	AAAGATGTGG	CCCCCTAAGC	TCCACGGGCT	CCCATTGCTA
12201	GGATTCTCT	GGGACGACAT	AGACGACAGC	GTGTTTTCCC	CGCAACCGCA
	CCTAAGGAGA	CCCTGCTGTA	TCTGCTGTCT	CACAAAAGGG	GCGTTGGCGT

Figure 27 M

12301 AGGAAAGCTT CCGCAGGCCA AGCAGCTTGT CCGATCTAGG CGCTGCGGCC  
TCCTTTCGAA GCGTCCGGT TCGTCGAACA GGCTAGATCC GCGACGCCGG

12351 CCGCGGTCAG ATGCTAGTAG CCCATTCCA AGCTTGATAG GGTCTCTTAC  
GGCGCCAGTC TACGATCATC GGGTAAAGGT TCGAACTATC CCAGAGAATG

12401 CAGCACTCGC ACCACCCGCC CGCGCCTGCT GGGCGAGGAG GAGTACCTAA  
GTCGTGAGCG TGGTGGGCGG GCGCGGACGA CCCGCTCCTC CTCATGGATT

12451 ACAACTCGCT GCTGCAGCCG CAGCGCGAAA AAAACCTGCC TCCGGCATTT  
TGTTGAGCGA CGACGTCGGC GTCGCGCTTT TTTTGGACGG AGGCCGTAAA

12501 CCCAACACG GGATAGAGAG CCTAGTGGAC AAGATGAGTA GATGGAAGAC  
GGGTGTTGTC CCTATCTCTC GGATCACCTG TTCTACTCAT CTACCTTCTG

12551 GTACGCGCAG GAGCACAGGG ACGTGCCAGG CCCGCGCCCG CCCACCCGTC  
CATGCGCGTC CTCGTGTCCC TGCACGGTCC GGGCGCGGGC GGGTGGGACG

12601 GTCAAAGGCA CGACCGTCAG CGGGGTCTGG TGTGGGAGGA CGATGACTCG  
CAGTTTCCGT GCTGGCAGTC GCCCCAGACC ACACCTCCT GCTACTGAGC

12651 GCAGACGACA GCAGCGTCCT GGATTTGGGA GGGAGTGGCA ACCCGTTTGC  
CGTCTGCTGT CGTCGCAGGA CCTAAACCTT CCTCACCGT TGGGCAAACG

12701 GCACCTTCGC CCCAGGCTGG GGAGAATGTT TTAACAAAAA AAAAAGCATG  
CGTGGAAGCG GGGTCCGACC CCTCTTACAA AATTTTTTTT TTTTTCGTAC

12751 ATGCAAAATA AAAAATCAC CAAGGCCATG GCACCGAGCG TTGGTTTCT  
TACGTTTTAT TTTTGTAGTG GTTCCGGTAC CGTGGCTCGC AACCAAAAGA

12801 TGTATTCCCC TTAGTATGCG GCGCGCGGCG ATGTATGAGG AAGGTCCTCC  
ACATAAGGGG AATCATACGC CGCGCGCCGC TACATACTCC TTCCAGGAGG

12851 TCCCTCCTAC GAGAGTGTGG TGAGCGCGGC GCCAGTGGCG GCGCGCTGG  
AGGGAGGATG CTCTCACACC ACTCGCGCCG CGGTCACCGC CGCCGCGACC

12901 GTTCTCCCTT CGATGCTCCC CTGGACCCGC CGTTTGTGCC TCCGCGGTAC  
CAAGAGGGAA GCTACGAGGG GACCTGGGCG GCAAACACGG AGGCGCCATG

12951 CTGCGGCCCTA CCGGGGGGAG AAACAGCATC CGTTACTCTG AGTTGGCACC  
GACGCCGGAT GGCCCCCTC TTTGTCTAG GCAATGAGAC TCAACCGTGG

13001 CCTATTCGAC ACCACCCGTG TGTACCTGGT GGACAACAAG TCAACGGATG  
GGATAAGCTG TGGTGGGCAC ACATGGACCA CCTGTTGTTT AGTTGCCTAC

13051 TGGCATCCCT GAACTACCAG AACGACCACA GCAACTTTCT GACCACGGTC  
ACCGTAGGGA CTTGATGGTC TTGCTGGTGT CGTTGAAAGA CTGGTGCCAG

13101 ATTCAAAACA ATGACTACAG CCCGGGGGAG GCAAGCACAC AGACCATCAA  
TAAGTTTGT TACTGATGTC GGGCCCCCTC CGTTCGTGTG TCTGGTAGTT

13151 TCTTGACGAC CGGTCGCACT GGGGCGGCGA CCTGAAAACC ATCCTGCATA  
AGAACTGCTG GCCAGCGTGA CCCC GCCGCT GGACTTTTGG TAGGACGTAT

Figure 27N

13251 CGGGTGATGG TGTGCGCCTT GCCTACTAAG GACAATCAGG TGGAGCTGAA  
 GCCCACTACC ACAGCGCGAA CGGATGATTC CTGTTAGTCC ACCTCGACTT  
 13301 ATACGAGTGG GTGGAGTTCA CGCTGCCCCG GGGCAACTAC TCCGAGACCA  
 TATGCTCACC CACCTCAAGT GCGACGGGCT CCCGTTGATG AGGCTCTGGT  
 13351 TGACCATAGA CCTTATGAAC AACGCGATCG TGGAGCACTA CTTGAAAGTG  
 ACTGGTATCT GGAATACTTG TTGCGCTAGC ACCTCGTGAT GAACTTTTCA  
 13401 GGCAGACAGA ACGGGGTTCT GGAAAGCGAC ATCGGGGTAA AGTTTGACAC  
 CCGTCTGTCT TGCCCCAAGA CCTTTCGCTG TAGCCCCATT TCAAAGTG  
 13451 CCGCAACTTC AGACTGGGGT TTGACCCCGT CACTGGTCTT GTCATGCCTG  
 GCGGTTGAAG TCTGACCCCA AACTGGGGCA GTGACCAGAA CAGTACGGAC  
 13501 GGGTATATAC AAACGAAGCC TTCCATCCAG ACATCATTTT GCTGCCAGGA  
 CCCATATATG TTTGCTTCGG AAGGTAGGTC TGTAAGTAAA CGACGGTCTT  
 13551 TGCGGGGTGG ACTTCACCCA CAGCCGCCTG AGCAACTTGT TGGGCATCCG  
 ACGCCCCACC TGAAGTGGGT GTCGGCGGAC TCGTTGAACA ACCCGTAGGC  
 13601 CAAGCGGCAA CCCTTCCAGG AGGGCTTTAG GATCACCTAC GATGATCTGG  
 GTTCGCCGTT GGGAAAGTCC TCCCGAAATC CTAGTGGATG CTACTAGACC  
 13651 AGGGTGGTAA CATTCCCACA CTGTTGGATG TGGACGCCTA CCAGGCGAGC  
 TCCCACCATT GTAAGGGCGT GACAACCTAC ACCTGCGGAT GGTCCGCTCG  
 13701 TTGAAAGATG ACACCGAACA GGGCGGGGGT GGCGCAGGCG GCAGCAACAG  
 AACTTTCTAC TGTGGCTTGT CCCGCCCCCA CCGCGTCCGC CGTCGTTGTC  
 13751 CAGTGGCAGC GGCGCGGAAG AGAACTCCAA CGCGGCAGCC GCGGCAATGC  
 GTCACCGTCG CCGCGCCTTC TCTTGAGGTT GCGCCGTCGG CGCCGTTACG  
 13801 AGCCGGTGGA GGACATGAAC GATCATGCCA TTCGCGGCGA CACCTTTGCC  
 TCGGCCACCT CCTGTACTTG CTAGTACGGT AAGCGCCGCT GTGGAAACGG  
 13851 ACACGGGCTG AGGAGAAGCG CGCTGAGGCC GAAGCAGCGG CCGAAGCTGC  
 TGTGCCCCGAC TCCTCTTCGC GCGACTCCGG CTTCGTCGCC GGCTTCGACG  
 13901 CGCCCCCGCT GCGCAACCCG AGGTCGAGAA GCCTCAGAAG AAACCGGTGA  
 GCGGGGGCGA CGCGTTGGGC TCCAGCTCTT CGGAGTCTTC TTTGGCCACT  
 13951 TCAAACCCCT GACAGAGGAC AGCAAGAAAC GCAGTTACAA CCTAATAAGC  
 AGTTTGGGGA CTGTCTCCTG TCGTTCCTTG CGTCAATGTT GGATTATTCG  
 14001 AATGACAGCA CCTTCACCCA GTACCGCAGC TGGTACCTTG CATACAACTA  
 TTAATGTCGT GGAAGTGGGT CATGGCGTCG ACCATGGAAC GTATGTTGAT  
 14051 CGGCGACCCCT CAGACCGGAA TCCGCTCATG GACCCTGCTT TGCACTCCTG  
 GCCGCTGGGA GTCTGGCCTT AGGCGAGTAC CTGGGACGAA ACGTGAGGAC  
 14101 ACGTAACCTG CGGCTCGGAG CAGGTCTACT GGTGCTTGCC AGACATGATG  
 TGCATTGGAC GCCGAGCCTC GTCCAGATGA CCAGCAACGG TCTGTACTAC

Figure 270

14201 GGTGGGCGCC GAGCTGTTGC CCGTGCAC TC CAAGAGCTTC TACAACGACC  
CCACCCGCGG CTCGACAACG GGCACGTGAG GTTCTCGAAG ATGTTGCTGG

14251 AGGCCGCTCTA CTCCCAACTC ATCCGCCAGT TTACCTCTCT GACCCACGTG  
TCCGGCAGAT GAGGGTTGAG TAGGCGGTCA AATGGAGAGA CTGGGTGCAC

14301 TTCAATCGCT TTCCCAGAGAA CCAGATTTTG GCGCGCCCGC CAGCCCCAC  
AAGTTAGCGA AAGGGCTCTT GGTCTAAAAC CGCGCGGGCG GTGCGGGGTG

14351 CATCACCACC GTCAGTGAAA ACGTTCCTGC TCTCACAGAT CACGGGACGC  
GTAGTGGTGG CAGTCACTTT TGCAAGGACG AGAGTGCTA GTGCCCTGCG

14401 TACCGCTGCG CAACAGCATC GGAGGAGTCC AGCGAGTGAC CATTACTGAC  
ATGGCGACGC GTTGTGCTAG CCTCCTCAGG TCGCTCACTG GTAATGACTG

14451 GCCAGACGCC GCACCTGCCC CTACGTTTAC AAGGCCCTGG GCATAGTCTC  
CGGTCTGCGG CGTGGACGGG GATGCAAATG TTCCGGGACC CGTATCAGAG

14501 GCCGCGCGTC CTATCGAGCC GCACTTTTTG AGCAAGCATG TCCATCCTTA  
CGGCGCGCAG GATAGCTCGG CGTGAAAAAC TCGTTCGTAC AGGTAGGAAT

14551 TATCGCCCGC CAATAACACA GGCTGGGGCC TCGCTTCCC AAGCAAGATG  
ATAGCGGGTC GTTATTGTGT CCGACCCCGG ACGCGAAGGG TTCGTTCTAC

14601 TTTGGCGGGG CCAAGAAGCG CTCCGACCAA CACCCAGTGC GCGTGCGCGG  
AAACCGCCCC GGTCTTTCG CAGGCTGGTT GTGGGTCACG CGCACGCGCC

14651 GCACTACCGC GCGCCCTGGG GCGCGCACAA ACGCGGCCGC ACTGGGCGCA  
CGTGATGGCG CGCGGGACCC CGCGCGTGTT TGCGCCGGCG TGACCCGCGT

14701 CCACCGTCGA TGACGCCATC GACGCGGTGG TGGAGGAGGC GCGCAACTAC  
GGTGGCAGCT ACTGCGGTAG CTGCGCCACC ACCTCCTCCG CGCGTTGATG

14751 ACGCCACGC CGCCACCAGT GTCCACAGTG GACGCGGCCA TTCAGACCGT  
TGCGGGTGCG GCGGTGGTCA CAGGTGTCAC CTGCGCCGGT AAGTCTGGCA

14801 GGTGCGCGGA GCGGCGCGCT ATGCTAAAAT GAAGAGACGG CGGAGGCGCG  
CCACGCGCCT CGGGCCGCGA TACGATTTTA CTTCTCTGCC GCCTCCGCGC

14851 TAGCACGTCTG CCACCGCCGC CGACCCGGCA CTGCGGCCCA ACGCGCGGCG  
ATCGTGCAGC GGTGGCGGCG GCTGGGCCGT GACGGCGGGT TGCGCGCCGC

14901 GCGGCCCTGC TTAACCGCGC ACGTCGCACC GGCCGACGGG CGGCCATGCG  
CGCCGGGACG AATTGGCGCG TGCAGCGTGG CCGGCTGCCC GCCGGTACGC

14951 GGCCGCTCGA AGGCTGGCCG CGGGTATTGT CACTGTGCCC CCCAGGTCCA  
CCGGCGAGCT TCCGACCGGC GCCCATAACA GTGACACGGG GGGTCCAGGT

15001 GGCAGCAGAGC GGCCGCCGCA GCAGCCGCGG CCATTAGTGC TATGACTCAG  
CCGCTGCTCG CCGGCGGCGT CGTCGGCGCC GGTAAATCACG ATACTGAGTC

15051 GGTGCGAGGG GCAACGTGTA TTGGGTGCGC GACTCGGTTA GCGGCCCTGC  
CCAGCGTCCC CGTTGCACAT AACCACGCG CTGAGCCAAT CGCCGGACGC

Figure 27P

15151 ACTTAGACTC GTACTGTTGT ATGTATCCAG CGGCGGCGGC GCGCAACGAA  
TGAATCTGAG CATGACAACA TACATAGGTC GCCGCCGCCG CGCGTTGCTT

15201 GCTATGTCCA AGCGCAAAAT CAAAGAAGAG ATGCTCCAGG TCATCGCGCC  
CGATACAGGT TCGCGTTTTA GTTCTTCTC TACGAGGTCC AGTAGCGCGG

15251 GGAGATCTAT GGCCCCCGA AGAAGGAAGA GCAGGATTAC AAGCCCCGAA  
CCTCTAGATA CCGGGGGGCT TCTTCCTTCT CGTCCTAATG TTCGGGGGCTT

15301 AGCTAAAGCG GGTCAAAAAG AAAAGAAAG ATGATGATGA TGAACCTGAC  
TCGATTTCCG CCAGTTTTTC TTTTCTTTC TACTACTACT ACTTGAAC TG

15351 GACGAGGTGG AACTGCTGCA CGCTACCGCG CCCAGGCGAC GGGTACAGTG  
CTGCTCCACC TTGACGACGT GCGATGGCGC GGGTCCGCTG CCCATGTAC

15401 GAAAGGTCTGA CGCGTAAAC GTGTTTTCG ACCCGGCACC ACCGTAGTCT  
CTTTCAGCT GCGCATTTTG CACAAAACGC TGGGCCGTGG TGGCATCAGA

15451 TTACGCCCCG TGAGCGCTCC ACCCGCACCT ACAAGCGCGT GTATGATGAG  
AATGCGGGCC ACTCGCGAGG TGGCGTGGA TGTTGCGCA CATACTACTC

15501 GTGTACGGCG ACGAGGACCT GCTTGAGCAG GCCAACGAGC GCCTCGGGGA  
CACATGCCGC TGCTCCTGGA CGAACTCGTC CGGTGCTCG CGGAGCCCT

15551 GTTTCCTTAC GGAAAGCGGC ATAAGGACAT GCTGGCGTTG CCGCTGGACG  
CAAACGGATG CCTTTCGCCG TATTCCTGTA CGACCGCAAC GGCGACCTGC

15601 AGGGCAACCC AACACCTAGC CTAAAGCCCG TAACACTGCA GCAGGTGCTG  
TCCCGTTGGG TTGTGGATCG GATTTCGGGC ATTGTGACGT CGTCCACGAC

15651 CCCGCGCTTG CACCGTCCGA AGAAAAGCGC GGCCTAAAGC GCGAGTCTGG  
GGGCGGAAC GTGGCAGGCT TCTTTTCGCG CCGGATTTG CGCTCAGACC

15701 TGACTTGCCA CCCACCGTGC AGCTGATGGT ACCCAAGCGC CAGCGACTGG  
ACTGAACCGT GGGTGGCAGC TCGACTACCA TGGGTTCGCG GTCGCTGACC

15751 AAGATGTCTT GGAAAAATG ACCGTGGAAC CTGGGCTGGA GCCCGAGGTC  
TTCTACAGAA CCTTTTTTAC TGGCACCTTG GACCCGACCT CGGGCTCCAG

15801 CGCGTGCGGC CAATCAAGCA GGTGGCGCCG GGAAGTGGCG TGCAGACCGT  
GCGCACGCCG GTTAGTTCGT CCACCGCGGC CCTGACCCGC ACGTCTGGCA

15851 GGACGTTTCA ATACCCACTA CCAGTAGCAC CAGTATTGCC ACCGCCACAG  
CCTGCAAGTC TATGGGTGAT GGTTCATCGTG GTCATAACGG TGGCGGTGTC

15901 AGGGCATGGA GACACAAACG TCCCCGGTTG CCTCAGCGGT GGCGGATGCC  
TCCCGTACCT CTGTGTTTGC AGGGGCCAAC GGAGTCGCCA CCGCCTACGG

15951 GCGGTGCAGG CCGTGCCTGC GGCCGCGTCC AAGACCTCTA CGGAGGTGCA  
CGCCACGTCC GCCAGCGACG CCGGCGCAGG TTCTGGAGAT GCCTCCACGT

16001 AACGGACCCG TGGATGTTTC GCGTTTCAGC CCCCCGGCGC CCGCGCCGTT  
TTGCCGCGGC ACCTACAAAG CGCAAAGTCG GGGGGCCGCG GCGCGGCGAA

Figure 27Q



16051 CGAGGAAGTA CGGCGCCGCC AGCGCGCTAC TGCCCGAATA TGCCCTACAT  
GCTCCTTCAT GCCGCGGCGG TCGCGCGATG ACGGGCTTAT ACGGGATGTA

16101 CCTTCCATTG CGCCTACCCC CGGCTATCGT GGCTACACCT ACCGCCCCAG  
GGAAGGTAAC GCGGATGGGG GCCGATAGCA CCGATGTGGA TGGCGGGGTG

16151 AAGACGAGCA ACTACCCGAC GCCGAACCAC CACTGGAACC CGCCGCCGCC  
TTCTGCTCGT TGATGGGCTG CGGCTTGGTG GTGACCTTGG GCGGCGGCGG

16201 GTCGCCGTCG CCAGCCCGTG CTGGCCCCGA TTTCCGTGCG CAGGGTGGCT  
CAGCGGCAGC GGTGCGGCAC GACCGGGGCT AAAGGCACGC GTCCACCGA

16251 CGCGAAGGAG GCAGGACCCT GGTGCTGCCA ACAGCGCGCT ACCACCCCAG  
GCGCTTCCTC CGTCCTGGGA CCACGACGGT TGTGCGCGCA TGGTGGGGTG

16301 CATCGTTTAA AAGCCGGTCT TTGTGGTTCT TGCAGATATG GCCCTCACCT  
GTAGCAAATT TTCGGCCAGA AACACCAAGA ACGTCTATAC CGGGAGTGGG

16351 GCCGCCTCCG TTTCCCGGTG CCGGGATTCC GAGGAAGAAT GCACCGTAGG  
CGGCGGAGGC AAAGGGCCAC GGCCCTAAGG CTCCTTCTTA CGTGGCATCC

16401 AGGGGCATGG CCGGCCACGG CCTGACGGG GGCATGCGTC GTGCGACCA  
TCCCCGTACC GGCCGGTGCC GGA CTGCCCCG CCGTACGCAG CACGCGTGGT

16451 CCGGCGGCGG CGCGCGTCGC ACCGTCGCAT GCGCGGCGGT ATCCTGCCCC  
GGCCGCCGCC GCGCGCAGCG TGGCAGCGTA CCGCCGCCA TAGGACGGGG

16501 TCCTTATTCC ACTGATCGCC GCGGCGATTG GCGCCGTGCC CGGAATTGCA  
AGGAATAAGG TGA CTAGCGG CGCCGCTAAC CCGGCGACGG GCCTTAACGT

16551 TCCGTGGCCT TGCAGGCGCA GAGACACTGA TTA AAAACAA GTTGCATGTG  
AGGCACCGGA ACGTCCGCGT CTCTGTGACT AATTTTGTG CAACGTACAC

16601 GAAAAATCAA AATAAAAAGT CTGGACTCTC ACGCTCGCTT GGTCTGTAA  
CTTTT TAGTT TTATTTTCA GACCTGAGAG TGCAGAGCGAA CCAGGACATT

16651 CTATTTTGTA GAATGGAAGA CATCAACTTT GCGTCTCTGG CCCC GCGACA  
GATAAAACAT CTTACCTTCT GTAGTTGAAA CGCAGAGACC GGGGCGCTGT

16701 CGGCTCGCGC CCGTTCATGG GAAACTGGCA AGATATCGGC ACCAGCAATA  
GCCGAGCGCG GGCAAGTACC CTTTGACCGT TCTATAGCCG TGGTCGTTAT

16751 TGAGCGGTGG CGCCTTCAGC TGGGGCTCGC TGTGGAGCGG CATTAAAAAT  
ACTCGCCACC GCGGAAGTCG ACCCCGAGCG ACACCTCGCC GTAATTTTTA

16801 TTCGGTTCCA CCGTTAAGAA CTATGGCAGC AAGGCCTGGA ACAGCAGCAC  
AAGCCAAGGT GGCAATTCTT GATACCGTCG TTCCGGACCT TGTGTCGCTG

16851 AGGCCAGATG CTGAGGGATA AGTTGAAAGA GCAAAATTTT CAACAAAAGG  
TCCGGTCTAC GACTCCCTAT TCAACTTTCT CGTTTAAAG GTTGTTTTCC

16901 TGGTAGATGG CCTGGCCTCT GGCATTAGCG GGGTGGTGGG CCTGGCCAAC  
ACCATCTACC GGACCGGAGA CCGTAATCGC CCCACCACCT GGACCGGTTG

16951 CAGGCAGTGC AAAATAAGAT TAACAGTAAG CTTGATCCCC GCCCTCCCGT  
GTCCGTCACG TTTTATTCTA ATTGTCATTC GAACTAGGGG CGGGAGGGCA

Figure 27R

17051 AAAAGCGTCC GCGCCCCGAC AGGGAAGAAA CTCTGGTGAC GCAAATAGAC  
TTTTTCGCAGG CGCGGGGCTG TCCCTTCTTT GAGACCACTG CGTTTATCTG

17101 GAGCCTCCCT CGTACGAGGA GGCCTAAAG CAAGGCCTGC CCACCACCCG  
CTCGGAGGGA GCATGCTCCT CCGTGATTTC GTTCCGGACG GGTGGTGGG

17151 TCCCATCGCG CCCATGGCTA CCGGAGTGCT GGGCCAGCAC ACACCCGTAA  
AGGGTAGCGC GGGTACCGAT GGCCTCACGA CCCGGTCGTG TGTGGGCATT

17201 CGCTGGACCT GCCTCCCCC GCCGACACCC AGCAGAAACC TGTGCTGCCA  
GCGACCTGGA CGGAGGGGGG CGGCTGTGGG TCGTCTTTGG ACACGACGGT

17251 GGCCCGACCG CCGTTGTGTG AACCCGTCCT AGCCGCGCGT CCCTGCGCCG  
CCGGGCTGGC GGCAACAACA TTGGGCAGGA TCGGCGCGCA GGGACGCGG

17301 CGCCGCCAGC GGTCCGCGAT CGTTGCGGCC CGTAGCCAGT GGCAACTGGC  
GCGGCGGTG CCAGGCGCTA GCAACGCCGG GCATCGGTCA CCGTTGACCG

17351 AAAGCACACT GAACAGCATC GTGGGTCTGG GGGTGCAATC CCTGAAGCGC  
TTTCGTGTGA CTTGTCTAG CACCCAGACC CCCACGTTAG GGACTTCGCG

17401 CGACGATGCT TCTGATAGCT AACGTGTCGT ATGTGTGTCA TGTATGCGTC  
GCTGCTACGA AGACTATCGA TTGCACAGCA TACACACAGT ACATACGCG

17451 CATGTCGCCC CCAGAGGAGC TGCTGAGCCG CCGCGCGCCC GCTTTCCAAG  
GTACAGCGGC GGTCTCCTCG ACGACTCGGC GCGCGCGGG CGAAAGGTT

17501 ATGGCTACCC CTTGATGAT GCCGCACTGG TCTTACATGC ACATCTCGGG  
TACCGATGGG GAAGCTACTA CGGCGTCACC AGAATGTACG TGTAGAGCCC

17551 CCAGGACGCC TCGGAGTACC TGAGCCCCGG GCTGGTGCA G TTTGCCCCGG  
GGTCTGCGG AGCCTCATGG ACTCGGGGCC CGACCACGTC AAACGGGCGC

17601 CCACCAGAC GTACTTCAGC CTGAATAACA AGTTTAGAAA CCCCACGGTG  
GGTGGCTCTG CATGAAGTCG GACTTATTGT TCAAATCTTT GGGGTGCCAC

17651 GCGCCTACGC ACGACGTGAC CACAGACCG TCCCAGCGTT TGACGCTGCG  
CGCGGATGCG TGCTGCACTG GTGTCTGGCC AGGGTCGCAA ACTGCGACGC

17701 GTTCATCCCT GTGGACCGTG AGGATACTGC GTACTCGTAC AAGGCGCGGT  
CAAGTAGGGA CACCTGGCAC TCCTATGACG CATGAGCATG TTCCGCGCCA

17751 TCACCCTAGC TGTGGGTGAT AACCGTGTGC TGGACATGGC TTCCACGTAC  
AGTGGGATCG ACACCCACTA TTGGCACACG ACCTGTACCG AAGGTGCATG

17801 TTTGACATCC GCGGCGTGCT GGACAGGGGC CCTACTTTTA AGCCCTACTC  
AAACTGTAGG CGCCGCACGA CCTGTCCCCG GGATGAAAAT TCGGGATGAG

17851 TGGCACTGCC TACAACGCCC TGGCTCCCAA GGGTGCCCCA AATCCTTGCG  
ACCGTGACGG ATGTTGCGGG ACCGAGGGTT CCCACGGGGT TTAGGAACGC

17901 AATGGGATGA AGCTGCTACT GCTCTTGAAT TAAACCTAGA AGAAGAGGAC  
TTACCCTACT TCGACGATGA CGAGAACTTT ATTTGGATCT TCTTCTCCTG

Figure 275

17951 GATGACAACG AAGACGAAGT AGACGAGCAA GCTGAGCAGC AAAAAACTCA  
CTACTGTTGC TTCTGCTTCA TCTGCTCGTT CGACTCGTCG TTTTTTGAGT

18001 CGTATTTTGGG CAGGCGCCTT ATTCTGGTAT AAATATTACA AAGGAGGGTA  
GCATAAACCC GTCCGCGGAA TAAGACCATA TTTATAATGT TTCCTCCCAT

18051 TTCAAATAGG TGTGCAAGGT CAAACACCTA AATATGCCGA TAAACATTT  
AAGTTTATCC ACAGCTTCCA GTTTGTGGAT TTATACGGCT ATTTTGTAAG

18101 CAACCTGAAC CTCAAATAGG AGAATCTCAG TGGTACGAAA CAGAAATTAA  
GTTGGACTTG GAGTTTATCC TCTTAGAGTC ACCATGCTTT GTCTTTAATT

18151 TCATGCAGCT GGGAGAGTCC TAAAAAAGAC TACCCCAATG AAACCATGTT  
AGTACGTCGA CCCTCTCAGG ATTTTTTCTG ATGGGGTTAC TTTGGTACAA

18201 ACGGTTTATA TGCAAAACCC ACAATGAAA ATGGAGGGCA AGGCATTCTT  
TGCCAAGTAT ACGTTTGGG GTTTTACTTT TACCTCCCGT TCCGTAAGAA

18251 GTAAAGCAAC AAAATGGAAA GCTAGAAAGT CAAGTGGAAA TGCAATTTTT  
CATTTTCGTTG TTTTACCTTT CGATCTTTCA GTTCACCTTT ACGTTAAAAA

18301 CTCAACTACT GAGGCAGCCG CAGGCAATGG TGATAACTTG ACTCCTAAAG  
GAGTTGATGA CTCCGTCGGC GTCCGTTACC ACTATTGAAC TGAGGATTTC

18351 TGGTATTGTA CAGTGAAGAT GTAGATATAG AAACCCAGCA CACTCATATT  
ACCATAACAT GTCACCTTCA CATCTATATC TTTGGGGTCT GTGAGTATAA

18401 TCTTACATGC CCACTATTAA GGAAGGTAAC TCACGAGAAC TAATGGGCCA  
AGAATGTACG GGTGATAATT CCTTCCATTG AGTGCTCTTG ATTACCCGGT

18451 ACAATCTATG CCCAACAGGC CTAATTACAT TGCTTTTAGG GACAATTTTA  
TGTTAGATAC GGGTTGTCCG GATTAATGTA ACGAAAATCC CTGTTAAAT

18501 TTGGTCTAAT GTATTACAAC AGCACGGGTA ATATGGGTGT TCTGGCGGGC  
AACCAGATTA CATAATGTTG TCGTGCCCAT TATACCCACA AGACCGCCCG

18551 CAAGCATCGC AGTTGAATGC TGTGTAGAT TTGCAAGACA GAAACACAGA  
GTTTCGTAGC TCAACTTACG ACAACATCTA AACGTTCTGT CTTTGTGTCT

18601 GCTTTCATAC CAGCTTTTGC TTGATTCCAT TGGTGATAGA ACCAGGTACT  
CGAAAGTATG GTCGAAAACG AACTAAGGTA ACCACTATCT TGGTCCATGA

18651 TTTCTATGTG GAATCAGGCT GTTGACAGCT ATGATCCAGA TGTTAGAATT  
AAAGATACAC CTTAGTCCGA CAACTGTCGA TACTAGGTCT ACAATCTTAA

18701 ATTGAAAAATC ATGGAAGTGA AGATGAACTT CCAAATTACT GCTTTCCTACT  
TAACTTTTAG TACCTTGACT TCTACTTGAA GGTTTAATGA CGAAAGGTGA

18751 GGGAGGTGTG ATTAATACAG AGACTCTTAC CAAGGTAAAA CCTAAACAG  
CCCTCCACAC TAATTATGTC TCTGAGAATG GTTCCATTTT GGATTTTGTC

18801 GTCAGGAAAA TGGATGGGAA AAAGATGCTA CAGAAATTTT AGATAAAAAAT  
CAGTCCTTTT ACCTACCCCT TTTCTACGAT GTCTTAAAAG TCTATTTTTA

18851 GAAATAAGAG TTGGAAATAA TTTTGCCATG GAAATCAATC TAAATGCCAA  
CTTTATTCTC AACCTTTATT AAAACGGTAC CTTTAGTTAG ATTTACGGTT

Figure 27T

18951 AGCTAAAGTA CAGTCCTTCC AACGTAAAAA TTTCTGATAA CCCAAACACC  
TCGATTTCAT GTCAGGAAGG TTGCATTTTT AAAGACTATT GGGTTTGTGG

19001 TACGACTACA TGAACAAGCG AGTGGTGGCT CCCGGGCTAG TGGACTGCTA  
ATGCTGATGT ACTTGTTCGC TCACCACCGA GGGCCCGATC ACCTGACGAT

19051 CATTAAACCTT GGAGCACGCT GGTCCCTTGA CTATATGGAC AACGTCAACC  
GTAATTGGAA CCTCGTGC GA CAGGGAACT GATATACCTG TTGCAGTTGG

19101 CATTTAACCA CCACCGCAAT GCTGGCCTGC GCTACCGCTC AATGTTGCTG  
GTAAATTGGT GGTGGCGTTA CGACCGGACG CGATGGCGAG TTACAACGAC

19151 GGCAATGGTC GCTATGTGCC CTTCCACATC CAGGTGCCTC AGAAGTTCTT  
CCGTTACCAG CGATACACGG GAAGGTGTAG GTCCACGGAG TCTTCAAGAA

19201 TGCCATTAAA AACCTCCTTC TCCTGCCGGG CTCATACACC TACGAGTGG A  
ACGGTAATTT TTGGAGGAAG AGGACGGCCC GAGTATGTGG ATGCTCACCT

19251 ACTTCAGGAA GGATGTAAAC ATGGTTCTGC AGAGCTCCCT AGGAAATGAC  
TGAAGTCCTT CCTACAATTG TACCAAGACG TCTCGAGGGA TCCTTTACTG

19301 CTAAGGGTTG ACGGAGCCAG CATTAAGTTT GATAGCATTT GCCTTTACGC  
GATTCCCAAC TGCCTCGGTC GTAATTCAAA CTATCGTAAA CGGAAATGCG

19351 CACCTTCTTC CCCATGGCCC ACAACACCGC CTCCACGCTT GAGGCCATGC  
GTGGAAGAAG GGGTACCGGG TGTGTGGCG GAGGTGCGAA CTCCGGTACG

19401 TTAGAAACGA CACCAACGAC CAGTCCTTTA ACGACTATCT CTCCGCCGCC  
AATCTTTGCT GTGGTTGCTG GTCAGGAAAT TGCTGATAGA GAGGCGGCGG

19451 AACATGCTCT ACCCTATACC CGCCAACGCT ACCAACGTGC CCATATCCAT  
TTGTACGAGA TGGGATATGG GCGGTTGCGA TGGTTGCACG GGTATAGGTA

19501 CCCCTCCCGC AACTGGGCGG CTTTCCGCGG CTGGGCCCTT ACGCGCCTTA  
GGGGAGGGCG GTGACCCGCC GAAAGGCGCC GACCCGGAAG TGCGCGGAAT

19551 AGACTAAGGA AACCCCATCA CTGGGCTCGG GCTACGACCC TTATTACACC  
TCTGATTCTT TTGGGGTAGT GACCCGAGCC CGATGCTGGG AATAATGTGG

19601 TACTCTGGCT CTATACCCTA CCTAGATGGA ACCTTTTACC TCAACCACAC  
ATGAGACCGA GATATGGGAT GGATCTACCT TGGAAAATGG AGTTGGTGTG

19651 CTTTAAGAAG GTGGCCATTA CTTTGACTC TTCTGTCAGC TGGCCTGGCA  
GAAATTCTTC CACCGGTAAT GGAAACTGAG AAGACAGTCG ACCGGACCGT

19701 ATGACCGCCT GCTTACCCCC AACGAGTTTG AAATTAAGCG CTCAGTTGAC  
TACTGGCGGA CGAATGGGGG TTGCTCAAAC TTTAATTCGC GAGTCAACTG

19751 GGGGAGGGTT ACAACGTTGC CCACTGTAAC ATGACCAAAG ACTGGTTTCT  
CCCCCTCCCA TGTGCAACG GGTCAATTG TACTGGTTTC TGACCAAGGA

19801 GGTACAAATG CTAGCTAACT ATAACATTGG CTACCAGGGC TTCTATATCC  
CCATGTTTAC GATCGATTGA TATTGTAAAC GATGGTCCCG AAGATATAGG

Figure 274

19851 CAGAGAGCTA CAAGGACCGC ATGTACTCCT TCTTTAGAAA CTTCCAGCCC  
GTCTCTCGAT GTTCCTGGCG TACATGAGGA AGAAATCTTT GAAGGTCGGG

19901 ATGAGCCGTC AGGTGGTGGG TGATACTAAA TACAAGGACT ACCAACAGGT  
TACTCGGCAG TCCACCACCT ACTATGATTT ATGTTCTTGA TGGTTGTCCA

19951 GGGCATCCTA CACCAACACA ACAACTCTGG ATTTGTTGGC TACCTTGCCC  
CCCGTAGGAT GTGGTTGTGT TGTGAGACC TAAACAACCG ATGGAACGGG

20001 CCACCATGCG CGAAGGACAG GCCTACCCTG CTAACCTCCC CTATCCGCTT  
GGTGGTACGC GCTTCTGTG CCGATGGGAC GATTGAAGGG GATAGGCGAA

20051 ATAGGCAAGA CCGCAGTTGA CAGCATTACC CAGAAAAAGT TTCTTTGCGA  
TATCCGTTCT GCGGTCAACT GTCGTAATGG GTCTTTTTC AAGAAACGCT

20101 TCGCACCCCTT TGGCGCATCC CATTCTCCAG TAACCTTATG TCCATGGGCG  
AGCGTGGGAA ACCGCGTAGG GTAAGAGGTC ATTGAAATAC AGGTACCCGC

20151 CACTCACAGA CCTGGGCCAA AACCTTCTCT ACGCCAACTC CGCCACGCG  
GTGAGTGTCT GGACCCGGTT TTGGAAGAGA TCGGTTGAG GCGGGTGCGC

20201 CTAGACATGA CTTTGTAGGT GGATCCCATG GACGAGCCCA CCCTTCTTTA  
GATCTGTACT GAAAACTCCA CCTAGGGTAC CTGCTCGGGT GGAAGAAAT

20251 TGTTTTGTGT GAAGTCTTTG ACGTGGTCCG TGTGCACCAG CCGCACCGCG  
ACAAAACAAA CTTCAGAAAC TGCACCAGGC ACACGTGGTC GGCGTGCGCG

20301 GCGTCATCGA AACCGTGTAC CTGCGCACGC CTTCTCGGC CGGCAACGCC  
CGCAGTAGCT TTGGCACATG GACGCGTGC GGAAGAGCCG GCCGTTGCGG

20351 ACAACATAAA GAAGCAAGCA ACATCAACAA CAGCTGCCGC CATGGGCTCC  
TGTTGTATTT CTTGTTTCGT TGTAGTTGTT GTCGACGGCG GTACCCGAGG

20401 AGTGAGCAGG AACTGAAAGC CATTGTCAA GATCTTGGTT GTGGGCCATA  
TCACTCGTCC TTGACTTTTC GTAACAGTTT CTAGAACCAA CACCCGGTAT

20451 TTTTTTGGGC ACCTATGACA AGCGCTTTCC AGGCTTTGTT TCTCCACACA  
AAAAAACCCG TGGATACTGT TCGCGAAAGG TCCGAAACAA AGAGGTGTGT

20501 AGCTCGCCTG CGCCATAGTC AATACGGCCG GTCGCGAGAC TGGGGGCGTA  
TCGAGCGGAC GCGGTATCAG TTATGCCGGC CAGCGCTCTG ACCCCCGCAT

20551 CACTGGATGG CCTTTGCCTG GAACCCGCAC TCAAAAACAT GCTACCTCTT  
GTGACCTACC GGAAACGGAC CTTGGGCGTG AGTTTTTGTA CGATGGAGAA

20601 TGAGCCCTTT GGCTTTTCTG ACCAGCGACT CAAGCAGGTT TACCAGTTTG  
ACTCGGGAAA CCGAAAAGAC TGGTCGCTGA GTTCGTCAA ATGGTCAAAC

20651 AGTACGAGTC ACTCCTGCGC CGTAGCGCCA TTGCTTCTTC CCCCACCGC  
TCATGCTCAG TGAGGACGCG GCATCGCGGT AACGAAGAAG GGGGCTGGCG

20701 TGTATAACGC TGGAAAAGTC CACCCAAAGC GTACAGGGGC CCAACTCGGC  
ACATATTGCG ACCTTTTTCAG GTGGGTTTCG CATGTCCCCG GGTGAGCCG

20751 CGCCTGTGGA CTATTCTGCT GCATGTTTCT CCACGCCTTT GCCAACTGGC  
GCGGACACCT GATAAGACGA CGTACAAAGA GGTGCGGAAA CGGTTGACCG

Figure 27 V.

20851 CCCAACTCCA TGCTCAACAG TCCCCAGGTA CAGCCCACCC TCGTTCGCAA  
GGGTTGAGGT ACGAGTTGTC AGGGGTCCAT GTCGGGTGGG ACGCAGCGTT

20901 CCAGGAACAG CTCTACAGCT TCCTGGAGCG CCACTCGCCC TACTTCCGCA  
GGTCCTTGTC GAGATGTCGA AGGACCTCGC GGTGAGCGGG ATGAAGGCGT

20951 GCCACAGTGC GCAGATTAGG AGCGCCACTT CTTTTGTCA CTTGAAAAAC  
CGGTGTCACG CGTCTAATCC TCGCGGTGAA GAAAAACAGT GAACTTTTTG

21001 ATGTAAAAAT AATGTACTAG AGACACTTTC AATAAAGGCA AATGCTTTTA  
TACATTTTAA TTACATGATC TCTGTGAAAG TTATTTCCGT TTACGAAAAAT

21051 TTTGTACACT CTCGGGTGAT TATTTACCCC CACCCTTGCC GTCTGCGCCC  
AAACATGTGA GAGCCCACTA ATAAATGGGG GTGGGAACGG CAGACGCGGC

21101 TTTAAAAATC AAAGGGGTTT TGCCGCGCAT CGCTATGCGC CACTGGCAGG  
AAATTTTGTAG TTTCCCCAAG ACGGCGCGTA GCGATACGCG GTGACCGTCC

21151 GACACGTTGC GATACTGGTG TTTAGTGCTC CACTTAAACT CAGGCACAAC  
CTGTGCAACG CTATGACCAC AAATCACGAG GTGAATTTGA GTCCGTGTTG

21201 CATCCGCGGC AGCTCGGTGA AGTTTTCACT CCACAGGCTG CGCACCATCA  
GTAGGCGCCG TCGAGCCACT TCAAAAAGTGA GGTGTCCGAC GCGTGGTAGT

21251 CCAACGCGTT TAGCAGGTCG GCGCCGATA TCTTGAAAGT GCAGTTGGGG  
GGTTGCGCAA ATCGTCCAGC CCGCGGCTAT AGAACTTCAG CGTCAACCCC

21301 CCTCCGCCCT GCGCGCGCGA GTTGCATAC ACAGGGTTGC AGCACTGGAA  
GGAGGCGGGA CGCGCGCGCT CAACGCTATG TGTCCCAACG TCGTGACCTT

21351 CACTATCAGC GCCGGGTGGT GCACGCTGGC CAGCACGCTC TTGTCCGAGA  
GTGATAGTCG CGGCCACCA CGTGCGACCG GTCGTGCGAG AACAGCCTCT

21401 TCAGATCCGC GTCCAGGTCC TCCGCGTTGC TCAGGGCGAA CGGAGTCAAC  
AGTCTAGGCG CAGGTCCAGG AGGCGCAACG AGTCCCGCTT GCCTCAGTTG

21451 TTTGGTAGCT GCCTTCCCAA AAAGGGCGCG TGCCCAGGCT TTGAGTTGCA  
AAACCATCGA CGGAAGGGTT TTTCCCGCGC ACGGGTCCGA AACTCAACGT

21501 CTCGCACCGT AGTGGCATCA AAAGGTGACC GTGCCCGGTC TGGGCGTTAG  
GAGCGTGGCA TCACCGTAGT TTTCCACTGG CACGGGCCAG ACCCGCAATC

21551 GATACAGCGC CTGCATAAAA GCCTTGATCT GCTTAAAAGC CACCTGAGCC  
CTATGTCGCG GACGTATTTT CGGAAGTAGA CGAATTTTCG GTGGACTCGG

21601 TTTGCGCCTT CAGAGAAGAA CATGCCGCAA GACTTGCCGG AAAACTGATT  
AAACGCGGAA GTCTCTTCTT GTACGGCGTT CTGAACGGCC TTTTGACTAA

21651 GGCCGGACAG GCCGCGTCGT GCACGCAGCA CCTTGCGTCG GTGTTGGAGA  
CCGGCCTGTC CGGCGCAGCA CGTGCGTCGT GGAACGCAGC CACAACCTCT

21701 TCTGCACCAC ATTTCCGCCC CACCGGTTCT TCACGATCTT GGCCTTGCTA  
AGACGTGGTG TAAAGCCGGG GTGGCCAAGA AGTGCTAGAA CCGGAACGAT

Figure 27 W

21801 AATCACGTGC TCCTTATTTA TCATAATGCT TCCGTGTAGA CACTTAAGCT  
TTAGTGCACG AGGAATAAAT AGTATTACGA AGGCACATCT GTGAATTCGA

21851 CGCCTTCGAT CTCAGCGCAG CGGTGCAGCC ACAACGCGCA GCCCGTGGGC  
GCGGAAGCTA GAGTCGCGTC GCCACGTCGG TGTTCGCGCT CGGGCACCCG

21901 TCGTGATGCT TGTAGGTCAC CTCTGCAAAC GACTGCAGGT ACGCCTGCAG  
AGCACTACGA ACATCCAGTG GAGACGTTG CTGACGTCCA TGCGGACGTC

21951 GAATCGCCCC ATCATCGTCA CAAAGGTCTT GTTGCTGGTG AAGGTCAGCT  
CTTAGCGGGG TAGTAGCAGT GTTTCAGAA CAACGACCAC TTCCAGTCGA

22001 GCAACCCGCG GTGCTCCTCG TTCAGCCAGG TCTTGCCATAC GGCCGCCAGA  
CGTTGGGCGC CACGAGGAGC AAGTCGGTCC AGAACGTATG CCGGCGGTCT

22051 GCTTCCACTT GGTCAGGCAG TAGTTTGAAG TTCGCCTTTA GATCGTTATC  
CGAAGGTGAA CCAGTCCGTC ATCAAACCTT AAGCGGAAAT CTAGCAATAG

22101 CACGTGGTAC TTGTCCATCA GCGCGCGCGC AGCCTCCATG CCCTTCTCCC  
GTGCACCATG AACAGGTAGT CGCGCGCGCG TCGGAGGTAC GGAAGAGGG

22151 ACGCAGACAC GATCGGCACA CTCAGCGGGT TCATCACCGT AATTTCACCT  
TGCCTCTGTG CTAGCCGTGT GAGTCGCCCA AGTAGTGGCA TTAAAGTGAA

22201 TCCGCTTCGC TGGGCTCTTC CTCTTCCTCT TCGGTCCGCA TACCACGCGC  
AGGCGAAGCG ACCCGAGAAG GAGAAGGAGA ACGCAGGCGT ATGGTGCAGC

22251 CACTGGGTCG TCTTCATTCA GCCGCCGCAC TGTGCGCTTA CCTCCTTTGC  
GTGACCCAGC AGAAGTAAGT CGGCGGCGTG ACACGCGAAT GGAGGAAACG

22301 CATGCTTGAT TAGCACCGGT GGGTTGCTGA AACCACCAT TTGTAGCGCC  
GTACGAAC TAATCGTGCCA CCAACGACT TTGGGTGGTA AACATCGCGG

22351 ACATCTTCTC TTTCTTCCTC GCTGTCCACG ATTACCTCTG GTGATGGCGG  
TGTAAGAG AAAGAAGGAG CGACAGGTGC TAATGGAGAC CACTACCGCC

22401 GCGCTCGGGC TTGGGAGAAG GCGCTTCTT TTTCTTCTTG GCGCAATGG  
CGCGAGCCCG AACCTCTTC CCGCAAGAA AAAGAAGAAC CCGGTTACC

22451 CCAAATCCGC CGCCGAGGTC GATGGCCGCG GGCTGGGTGT GCGCGGCACC  
GGTTTAGGCG GCGGCTCCAG CTACCGGCGC CCGACCCACA CGCGCGTGG

22501 AGCGCTCTT GTGATGAGTC TTCCTCGTCC TCGGACTCGA TACGCCGCCT  
TCGCGCAGAA CACTACTCAG AAGGAGCAGG AGCCTGAGCT ATGCGGCGGA

22551 CATCCGCTTT TTTGGGGGCG CCCGGGGAGG CGGCGGCGAC GGGGACGGG  
GTAGCGAAA AAACCCCGC GGGCCCTCC GCCGCGCTG CCCCTGCCCC

22601 ACGACACGTC CTCCATGGTT GGGGACGTC GCGCCGCACC GCGTCCGCGC  
TGCTGTGAG GAGGTACCAA CCCCCTGCAG CGCGGCGTGG CGCAGGCGC

22651 TCGGGGGTGG TTTCGCGCTG CTCCTCTTCC CGACTGGCCA TTTCTTCTC  
AGCCCCACC AAAGCGCGAC GAGGAGAAGG GCTGACCGGT AAAGGAAGAG

Figure 27X

22751 CCGCCCCCTC TGAGTTCGCC ACCACCGCCT CCACCGATGC CGCCAACGCG  
GGCGGGGGAG ACTCAAGCGG TGGTGGCGGA GGTGGCTACG GCGGTTGCGC

22801 CCTACCACCT TCCCCGTCGA GGCACCCCG CTTGAGGAGG AGGAAGTGAT  
GGATGGTGGA AGGGGCAGCT CCGTGGGGGC GAACTCCTCC TCCTTACTA

22851 TATCGAGCAG GACCCAGGTT TTGTAAGCGA AGACGACGAG GACCGCTCAG  
ATAGCTCGTC CTGGGTCCAA AACATTGCT TCTGCTGCTC CTGGCGAGTC

22901 TACCAACAGA GGATAAAAAG CAAGACCAGG ACAACGCAGA GGCAAACGAG  
ATGGTTGTCT CCTATTTTTC GTTCTGGTCC TGTTCGTCT CCGTTTGCTC

22951 GAACAAGTCG GGCGGGGGGA CGAAAGGCAT GGCGACTACC TAGATGTGGG  
CTTGTTACAG CCGCCCCCT GCTTTCGTA CCGCTGATGG ATCTACACCC

23001 AGACGACGTG CTGTTGAAGC ATCTGCAGCG CCAGTGCGCC ATTATCTGCG  
TCTGCTGCAC GACAACTTCG TAGACGTGCG GGTCACGCGG TAATAGACGC

23051 ACGCGTTGCA AGAGCGCAGC GATGTGCCCC TCGCCATAGC GGATGTCAGC  
TGCGCAACGT TCTCGCGTCG CTACACGGGG AGCGGTATCG CCTACAGTCG

23101 CTTGCCCTACG AACGCCACCT ATTCTACCG CGCGTACCCC CCAAACGCCA  
GAACGGATGC TTGCGGTGGA TAAGAGTGGC GCGCATGGGG GGTTTGCGGT

23151 AGAAAACGGC ACATGCGAGC CCAACCCGCG CCTCAACTTC TACCCCGTAT  
TCTTTTGCCG TGTACGCTCG GGTGGGCGC GGAGTTGAAG ATGGGGCATA

23201 TTGCCGTGCC AGAGGTGCTT GCCACCTATC ACATCTTTTT CCAAACCTGC  
AACGGCACGG TCTCCACGAA CGGTGGATAG TGTAGAAAAA GGTTTTGACG

23251 AAGATACCCC TATCCTGCCG TGCCAACCGC AGCCGAGCGG ACAAGCAGCT  
TTCTATGGGG ATAGGACGGC ACGGTTGGCG TCGGCTCGCC TGTTGCTCGA

23301 GGCCTTGCGG CAGGGCGCTG TCATACCTGA TATCGCCTCG CTCAACGAAG  
CCGGAACGCC GTCCCGCGAC AGTATGGACT ATAGCGGAGC GAGTTGCTTC

23351 TGCCAAAAAT CTTTGAGGGT CTTGGACGCG ACGAGAAGCG CGCGGCAAAC  
ACGGTTTTTA GAAACTCCCA GAACCTGCGC TGCTCTTCGC GCGCCGTTTG

23401 GCTCTGCAAC AGGAAAACAG CGAAAATGAA AGTCACTCTG GAGTGTGGT  
CGAGACGTTG TCCTTTTGTC GCTTTTACTT TCAGTGAGAC CTCACAACCA

23451 GGAACTCGAG GGTGACAACG CGCGCCTAGC CGTACTAAAA CGCAGCATCG  
CCTTGAGCTC CCACTGTTGC GCGCGGATCG GCATGATTTT GCGTCGTAGC

23501 AGGTACCCCA CTTTGCCCTAC CCGGCACTTA ACCTACCCCC CAAGGTCATG  
TCCAGTGGGT GAAACGGATG GGCCGTGAAT TGGATGGGGG GTTCCAGTAC

23551 AGCACAGTCA TGAGTGAGCT GATCGTGCGC CGTGCGCAGC CCCTGGAGAG  
TCGTGTCAGT ACTCACTCGA CTAGCACGCG GCACGCGTCG GGGACCTCTC

23601 GGATGCAAAT TTGCAAGAAC AAACAGAGGA GGGCCTACCC GCAGTTGGCG  
CCTACGTTTA AACGTTCTTG TTTGTCTCCT CCCGGATGGG CGTCAACCGC

Figure 27 Y



23701 GAGCGACGCA AACTAATGAT GGCCGCAGTG CTCGTTACCG TGGAGCTTGA  
CTCGCTGCGT TTGATTACTA CCGGCGTCAC GAGCAATGGC ACCTCGAACT

23751 GTGCATGCAG CGGTTCTTTG CTGACCCGGA GATGCAGCGC AAGCTAGAGG  
CACGTACGTC GCCAAGAAAC GACTGGGCCT CTACGTCGCG TTCGATCTCC

23801 AAACATTGCA CTACACCTTT CGACAGGGCT ACGTACGCCA GGCCTGCAAG  
TTTGTAACGT GATGTGGAAG GCTGTCCCGA TGCATGCGGT CCGGACGTTC

23851 ATCTCCAACG TGGAGCTCTG CAACCTGGTC TCCTACCTTG GAATTTTGCA  
TAGAGGTTGC ACCTCGAGAC GTTGGACCAG AGGATGGAAC CTTAAACGCT

23901 CGAAAACCGC CTTGGGCAAA ACGTGCTTCA TTCCACGCTC AAGGGCGAGG  
GCTTTTGCGG GAACCCGTTT TGCACGAAGT AAGGTGCGAG TTCCCGCTCC

23951 CGCGCCGCGA CTACGTCCGC GACTGCGTTT ACTTATTTCT ATGCTACACC  
GCGCGGCGCT GATGCAGGCG CTGACGCAAA TGAATAAAGA TACGATGTGG

24001 TGGCAGACGG CCATGGGCGT TTGGCAGCAG TGCTTGAGG AGTGCAACCT  
ACCGTCTGCC GGTACCCGCA AACCGTCGTC ACGAACCTCC TCACGTGGA

24051 CAAGGAGCTG CAGAACTGC TAAAGCAAAA CTTGAAGGAC CTATGGACGG  
GTTCTCGAC GTCTTTGACG ATTTCTGTTT GAACTTCCTG GATACCTGCC

24101 CCTTCAACGA GCGCTCCGTG GCCGCGCACC TGGCGGACAT CATTTTCCCC  
GGAAGTTGCT CGCGAGGCAC CGGCGCGTGG ACCGCCTGTA GTAAAGGGG

24151 GAACGCCTGC TTAAACCCCT GCAACAGGGT CTGCCAGACT TCACCAGTCA  
CTTGCGGACG AATTTTGGA CGTTGTCCCA GACGGTCTGA AGTGGTCAGT

24201 AAGCATGTTG CAGAACTTTA GGAACCTTAT CCTAGAGCGC TCAGGAATCT  
TTCGTACAAC GTCTTGAAAT CCTTGAAATA GGATCTCGCG AGTCCTTAGA

24251 TGCCCGCCAC CTGCTGTGCA CTTCTAGCG ACTTTGTGCC CATTAAGTAC  
ACGGGCGGTG GACGACACGT GAAGGATCGC TGAAACACGG GTAATTCATG

24301 CGCGAATGCC CTCCGCCGCT TTGGGGCCAC TGCTACCTTC TGCAGCTAGC  
GCGCTTACGG GAGGCGGCGA AACCCCGGTG ACGATGGAAG ACGTCGATCG

24351 CAACTACCTT GCCTACCACT CTGACATAAT GGAAGACGTG AGCGGTGACG  
GTTGATGGAA CGGATGGTGA GACTGTATTA CCTTCTGCAC TCGCCACTGC

24401 GTCTACTGGA GTGTCACTGT CGCTGCAACC TATGCACCCC GCACCGCTCC  
CAGATGACCT CACAGTGACA GCGACGTTGG ATACGTGGGG CGTGGCGAGG

24451 CTGGTTTGCA ATTGCGAGCT GCTTAACGAA AGTCAAATTA TCGGTACCTT  
GACCAAACGT TAAGCGTCGA CGAATTGCTT TCAGTTTAAT AGCCATGGAA

24501 TGAGCTGCAG GGTCCCTCGC CTGACGAAAA GTCCGCGGCT CCGGGGTTGA  
ACTCGACGTC CCAGGGAGCG GACTGCTTTT CAGGCGCCGA GGCCCCAAT

24551 AACTCACTCC GGGGCTGTGG ACGTCGGCTT ACCTTCGCAA ATTTGTACCT  
TTGAGTGAGG CCCCACACC TGCAGCCGAA TGGAAGCGTT TAAACATGGA

Figure 272

24601 GAGGACTACC ACGCCACGA GATTAGGTTT TACGAAGACC AATCCCGCCC  
CTCCTGATGG TCGGGGTGCT CTAATCCAAG ATGCTTCTGG TTAGGGCGGG

24651 GCCTAATGCG GAGCTTACCG CCTGCGTCAT TACCCAGGGC CACATTCTTG  
CGGATTACGC CTCGAATGGC GGACGCAGTA ATGGGTCCCG GTGTAAGAAC

24701 GCCAATTGCA AGCCATCAAC AAAGCCCGCC AAGAGTTTCT GCTACGAAAG  
CGGTTAACGT TCGGTAGTTG TTTCGGGCGG TTCTCAAAGA CGATGCTTTC

24751 GGACGGGGGG TTTACTTGGA CCCCCAGTCC GCGGAGGAGC TCAACCCAAT  
CCTGCCCCC AAATGAACCT GGGGGTCAGG CCGCTCCTCG AGTTGGGTTA

24801 CCCCCCGCCG CCGCAGCCCT ATCAGCAGCA GCGCGGGGCC CTTGCTTCCC  
GGGGGGCGGC GCGCTCGGGA TAGTCGTCGT CCGCGCCCGG GAACGAAGGG

24851 AGGATGGCAC CCAAAAAGAA GCTGCAGCTG CCGCCGCCAC CCACGGACGA  
TCCTACCGTG GGTTTTCTT CGACGTCGAC GCGCGCGGTG GGTGCTGCT

24901 GGAGGAATAC TGGGACAGTC AGGCAGAGGA GGTTTTGAC GAGGAGGAGG  
CCTCCTTATG ACCCTGTCAG TCCGTCTCCT CCAAAACCTG CTCCTCCTCC

24951 AGGACATGAT GGAAGACTGG GAGAGCCTAG ACGAGGAAGC TTCCGAGGTC  
TCCTGTACTA CCTTCTGACC CTCTCGGATC TGCTCCTTCG AAGGCTCCAG

25001 GAAGAGGTGT CAGACGAAAC ACCGTCACCC TCGGTGCGAT TCCCCTCGCC  
CTTCTCCACA GTCTGCTTTG TGGCAGTGGG AGCCAGCGTA AGGGGAGCGG

25051 GCGCCCCCAG AAATCGGCAA CCGGTTCCAG CATGGCTACA ACCTCCGCTC  
CCGCGGGGTC TTTAGCCGTT GGCCAAGGTC GTACCGATGT TGGAGGCGAG

25101 CTCAGGCGCC GCGGCACTG CCGGTTGCGC GACCCAACCG TAGATGGGAC  
GAGTCCGCGG CCGCCGTGAC GGGCAAGCGG CTGGGTTGGC ATCTACCCTG

25151 ACCACTGGAA CCAGGGCCGG TAAGTCCAAG CAGCCGCCGC CGTTAGCCCA  
TGGTGACCTT GGTCCCGGCC ATTCAGGTTT GTCGGCGGCG GCAATCGGGT

25201 AGAGCAACAA CAGCGCCAAG GCTACCGCTC ATGGCGCGGG CACAAGAAGC  
TCTCGTTGTT GTCGCGGTTT CGATGGCGAG TACCGCGCCC GTGTCTTGC

25251 CCATAGTTGC TTGCTTGCAA GACTGTGGGG GCAACATCTC CTTGCCCCGC  
GGTATCAACG AACGAACGTT CTGACACCCC CGTTGTAGAG GAAGCGGGCG

25301 CGCTTTCTTC TCTACCATCA CCGCGTGGCC TTCCCCGTA ACATCCTGCA  
GCGAAAGAAG AGATGGTAGT GCCGCACCGG AAGGGGGCAT GTAGGACGT

25351 TTACTACCGT CATCTCTACA GCCCATACTG CACCGGCGGC AGCGGCAGCA  
AATGATGGCA GTAGAGATGT CGGGTATGAC GTGGCCGCGG TCGCCGTCGT

25401 ACAGCAGCGG CCACACAGAA GCAAAGGCGA CCGGATAGCA AGACTCTGAC  
TGTCGTCGCC GGTGTGTCTT CGTTTCCGCT GGCTATCGT TCTGAGACTG

25451 AAAGCCCAAG AAATCCACAG CCGCGGCAGC AGCAGGAGGA GGAGCGCTGC  
TTTCGGGTTT TTTAGGTGTC GCCGCCGTCG TCGTCTCCTT CCTCGCGACG

25501 GTCTGGCGCC CAACGAACCC GTATCGACCC GCGAGCTTAG AAACAGGATT  
CAGACCGCGG GTTGCTTGGG CATAGCTGGG CGCTCGAATC TTTGTCCTAA

Figure 27 AA

25551 TTTCCCACTC TGTATGCTAT ATTTCAACAG AGCAGGGGCC AAGAACAAGA  
AAAGGGTGAG ACATACGATA TAAAGTTGTC TCGTCCCCGG TTCTTGTTCT

25601 GCTGAAAATA AAAACAGGT CTCTGCGATC CCTCACCCGC AGCTGCCTGT  
CGACTTTTAT TTTTGTCCA GAGACGCTAG GGAGTGGGCG TCGACGGACA

25651 ATCACAAAAG CGAAGATCAG CTTGCGCGCA CGCTGGAAGA CGCGGAGGCT  
TAGTGTTTTT GCTTCTAGTC GAAGCCGCGT GCGACCTTCT GCGCCTCCGA

25701 CTCTTCAGTA AATACTGCGC GCTGACTCTT AAGGACTAGT TTCGCGCCCT  
GAGAAGTCAT TTATGACGCG CGACTGAGAA TTCCTGATCA AAGCGCGGGA

25751 TTCTCAAATT TAAGCGCGAA AACTACGTCA TCTCCAGCGG CCACACCCGG  
AAGAGTTTAA ATTCGCGCTT TTGATGCAGT AGAGGTCGCC GGTGTGGGCC

25801 CGCCAGCACC TGTGTGCAGC GCCATTATGA GCAAGGAAAT TCCCACGCCC  
CGGTCGTTGG ACAACAGTCG CGGTAATACT CGTTCTTTTA AGGGTGCGGG

25851 TACATGTGGA GTTACCAGCC ACAAATGGGA CTTGCGGCTG GAGCTGCCCCA  
ATGTACACCT CAATGGTCGG TGTTTACCCT GAACGCCGAC CTCGACGGGT

25901 AGACTACTCA ACCCGAATAA ACTACATGAG CGCGGGACCC CACATGATAT  
TCTGATGAGT TGGGCTTATT TGATGTACTC GCGCCCTGGG GTGTACTATA

25951 CCCGGGTCAA CGGAATACGC GCCCACCAGG ACCGAATTCT CCTGGAACAG  
GGGCCCAGTT GCCTTATGCG CGGGTGCTT TGGCTTAAGA GGACCTTGTC

26001 GCGGCTATTA CCACCACACC TCGTAATAAC CTTAATCCCC GTAGTTGGCC  
CGCCGATAAT GGTGGTGTGG AGCATTATTG GAATTAGGGG CATCAACCGG

26051 CGCTGCCCTG GTGTACCAGG AAAGTCCCGC TCCCACCACT GTGGTACTTC  
GCGACGGGAC CACATGGTCC TTTCAGGGCG AGGGTGGTGA CACCATGAAG

26101 CCAGAGACGC CCAGGCCGAA GTTCAGATGA CTAAGTCAGG GGCGCAGCTT  
GGTCTCTGCG GTCCCGCTT CAAGTCTACT GATTGAGTCC CCGCGTCGAA

26151 GCGGGCGGCT TTCGTACAGG GGTGCGGTCG CCCGGGCAGG GTATAACTCA  
CGCCCGCCGA AAGCAGTGTC CCACGCCAGC GGGCCCGTCC CATATTGAGT

26201 CCTGACAATC AGAGGGCGAG GTATTCAGCT CAACGACGAG TCGGTGAGCT  
GGACTGTTAG TCTCCCGCTC CATAAGTCGA GTTGCTGCTC AGCCACTCGA

26251 CCTCGCTTGG TCTCCGTCCG GACGGGACAT TTCAGATCGG CGGCGCCGGC  
GGAGCGAACC AGAGGCAGGC CTGCCCTGTA AAGTCTAGCC GCCGCGGCCG

26301 CGCTCTTCAT TCACGCCTCG TCAGGCAATC CTAAGTCTGC AGACCTCGTC  
GCGAGAAGTA AGTGCGGAGC AGTCCGTTAG GATTGAGACG TCTGGAGCAG

26351 CTCTGAGCCG CGCTCTGGAG GCATTGGAAC TCTGCAATTT ATTGAGGAGT  
GAGACTCGGC GCGAGACCTC CGTAACCTTG AGACGTTAAA TAACTCCTCA

26401 TTGTGCCATC GGTCTACTTT AACCCCTTCT CGGGACCTCC CGGCCACTAT  
AACACGGTAG CCAGATGAAA TTGGGAAGA GCCCTGGAGG GCCGGTGATA

26451 CCGGATCAAT TTATTCCTAA CTTTGACGCG GTAAAGGACT CGGCGGACGG  
GGCCTAGTTA AATAAGGATT GAAACTGCGC CATTCCTGA GCCGCCTGCC

Figure 27 AB

26501 CTACGACTGA ATGTTAAGTG GAGAGGCAGA GCAACTGCGC CTGAAACACC  
GATGCTGACT TACAATTAC CTCTCCGTCT CGTTGACGCG GACTTTGTGG

26551 TGGTCCACTG TCGCCGCCAC AAGTGCTTTG CCCGCGACTC CGGTGAGTTT  
ACCAGGTGAC AGCGGCGGTG TTCACGAAAC GGGCGCTGAG GCCACTCAAA

26601 TGCTACTTTG AATTGCCCCG GGATCATATC GAGGGCCCCG CGCACGGCGT  
ACGATGAAAC TTAACGGGCT CCTAGTATAG CTCCCGGGCC GCGTGCCGCA

26651 CCGGCTTACC GCCCAGGGAG AGCTTGCCCC TAGCCTGATT CGGGAGTTTA  
GGCCGAATGG CGGGTCCCTC TCGAACGGGC ATCGGACTAA GCCCTCAAAT

26701 CCCAGCGCCC CCTGCTAGTT GAGCGGGACA GGGGACCCTG TGTCTCTACT  
GGGTGCGGGG GGACGATCAA CTCGCCCTGT CCCCTGGGAC ACAAGAGTGA

26751 GTGATTTGCA ACTGTCCTAA CCCTGGATTA CATCAAGATC TTTGTTGCCA  
CACTAAACGT TGACAGGATT GGGACCTAAT GTAGTTCTAG AAACAACGGT

26801 TCTCTGTGCT GAGTATAATA AATACAGAAA TTAAATATA CTGGGGCTCC  
AGAGACACGA CTCATATTAT TTATGTCTTT AATTTTATAT GACCCCGAGG

26851 TATCGCCATC CTGTAAACGC CACCGTCTTC ACCCGCCCAA GCAAACCAAG  
ATAGCGGTAG GACATTTGCG GTGGCAGAAG TGGGCGGGTT CGTTTGTTTC

26901 GCGAACCTTA CCTGGTACTT TTAACATCTC TCCCTCTGTG ATTTACAACA  
CGCTTGGAAT GGACCATGAA AATTGTAGAG AGGGAGACAC TAAATGTTGT

26951 GTTTC AACCC AGACGGAGTG AGTCTACGAG AGAACCTCTC CGAGCTCAGC  
CAAAGTTGGG TCTGCCTCAC TCAGATGCTC TCTTGAGAG GCTCGAGTCG

27001 TACTCCATCA GAAAAACAC CACCCTCCTT ACCTGCCGGG AACGTACGAG  
ATGAGGTAGT CTTTTTGTG GTGGGAGGAA TGGACGGCCC TTGCATGCTC

27051 TGCGTCACCG GCCGCTGCAC CACACCTACC GCCTGACCGT AAACCAGACT  
ACGCAGTGCG CGGCACGTG GTGTGGATGG CGGACTGGCA TTTGGTCTGA

27101 TTTCCGGAC AGACCTCAAT AACTCTGTTT ACCAGAACAG GAGGTGAGCT  
AAAAGGCCTG TCTGGAGTTA TTGAGACAAA TGGTCTTGTC CTCCACTCGA

27151 TAGAAAACCC TTAGGGTATT AGGCCAAAGG CGCAGCTACT GTGGGGTTTA  
ATCTTTTGGG AATCCCATAA TCCGGTTTCC GCGTCGATGA CACCCCAAAT

27201 TGAACAATTC AAGCAACTCT ACGGGCTATT CTAATTCAGG TTTCTCTAGA  
ACTTGTTAAG TTCGTTGAGA TGCCCGATAA GATTAAGTCC AAAGAGATCT

27251 ATCGGGGTTG GGGTTATTCT CTGTCTTGTG ATTCTCTTTA TTCTTATACT  
TAGCCCCAAC CCAATAAGA GACAGAACAC TAAGAGAAAT AAGAATATGA

27301 AACGCTTCTC TGCCTAAGGC TCGCCGCCCTG CTGTGTGCAC ATTTGCATTT  
TTGCGAAGAG ACGGATTCCG AGCGGCGGAC GACACACGTG TAAACGTAAA

27351 ATTGTCAGCT TTTTAAACGC TGGGGTCGCC ACCCAAGATG ATTAGGTACA  
TAACAGTCGA AAAATTGCG ACCCCAGCGG TGGGTCTTAC TAATCCATGT

27401 TAATCCTAGG TTTACTCACC CTTGCGTCAG CCCACGGTAC CACCCAAAAG  
ATTAGGATCC AAATGAGTGG GAACGCAGTC GGGTGCCATG GTGGGTTTTC

Figure 27AC

27451 GTGGATTTTA AGGAGCCAGC CTGTAATGTT ACATTTCGAG CTGAAGCTAA  
CACCTAAAAT TCCTCGGTGC GACATTACAA TGTAAGCGTC GACTTCGATT

27501 TGAGTGCACC ACTCTTATAA AATGCACCAC AGAACATGAA AAGCTGCTTA  
ACTCACGTGG TGAGAAATATT TTACGTGGTG TCTTGACTT TTCGACGAAT

27551 TTCGCCACAA AAACAAAATT GGCAAGTATG CTGTTTATGC TATTTGGCAG  
AAGCGGTGTT TTTGTTTTAA CCGTTCATAC GACAAATACG ATAAACCGTC

27601 CCAGGTGACA CTACAGAGTA TAATGTTACA GTTTTCCAGG GTAAAAGTCA  
GGTCCACTGT GATGTCTCAT ATTACAATGT CAAAAGGTCC CATTTTCAGT

27651 TAAAACTTTT ATGTATACTT TTCCATTTTA TGAAATGTGC GACATTACCA  
ATTTTGAAA TACATATGAA AAGGTAAAAT ACTTTACACG CTGTAATGGT

27701 TGTACATGAG CAAACAGTAT AAGTTGTGGC CCCCACAAAA TTGTGTGGAA  
ACATGTACTC GTTGTCATA TTCAACACCG GGGGTGTTTT AACACACCTT

27751 AACACTGGCA CTTTCTGCTG CACTGCTATG CTAATTACAG TGCTCGCTTT  
TTGTGACCGT GAAAGACGAC GTGACGATAC GATTAATGTC ACGAGCGAAA

27801 GGTCTGTACC CTACTCTATA TTAAATACAA AAGCAGACGC AGCTTTATTG  
CCAGACATGG GATGAGATAT AATTTATGTT TTCGTCTGCG TCGAAATAAC

27851 AGGAAAAGAA AATGCCTTAA TTTACTAAGT TACAAAGCTA ATGTCACCAC  
TCCTTTTCTT TTACGGAATT AAATGATTCA ATGTTTCGAT TACAGTGGTG

27901 TAACTGCTTT ACTCGCTGCT TGCAAAACAA ATTCAAAAAG TTAGCATTAT  
ATTGACGAAA TGAGCGACGA ACGTTTTGTT TAAGTTTTTC AATCGTAATA

27951 AATTAGAATA GGATTTAAAC CCCCCGGTCA TTTCTGCTC AATACCATTC  
TTAATCTTAT CCTAAATTG GGGGGCCAGT AAAGGACGAG TTATGGTAAG

28001 CCCTGAACAA TTGACTCTAT GTGGGATATG CTCCAGCGCT ACAACCTTGA  
GGGACTTGTT AACTGAGATA CACCCTATAC GAGGTCGCGA TGTTGGAAT

28051 AGTCAGGCTT CCTGGATGTC AGCATCTGAC TTTGGCCAGC ACCTGTCCCG  
TCAGTCCGAA GGACCTACAG TCGTAGACTG AAACCGGTCG TGGACAGGGC

28101 CGGATTTGTT CCAGTCCAAC TACAGCGACC CACCCTAACA GAGATGACCA  
GCCTAAACAA GGTCAAGTTG ATGTCGCTGG GTGGGATTGT CTCTACTGGT

28151 ACACAACCAA CGCGGCCGCC GCTACCGGAC TTACATCTAC CACAAATACA  
TGTGTTGGTT GCGCCGGCGG CGATGGCCTG AATGTAGATG GTGTTTATGT

28201 CCCCAAGTTT CTGCCTTTGT CAATAACTGG GATAACTTGG GCATGTGGTG  
GGGGTTCAAA GACGGAAACA GTTATTGACC CTATTGAACC CGTACACCAC

28251 GTTCTCCATA GCGCTTATGT TTGTATGCCT TATTATTATG TGGCTCATCT  
CAAGAGGTAT CGCGAATACA AACATACGGA ATAATAATAC ACCGAGTAGA

28301 GCTGCCTAAA GCGCAAACGC GCCCGACCAC CCATCTATAG TCCCATCATT  
CGACGGATTT CGCGTTTGCG CGGGCTGGTG GGTAGATATC AGGGTAGTAA

28351 GTGCTACACC CAAACAATGA TGGAAATCCAT AGATTGGACG GACTGAAACA  
CACGATGTGG GTTTGTACT ACCTTAGGTA TCTAACCTGC CTGACTTTGT

Figure 27A D

28451 TTTTATATTA CTGACCCTTG TTGCGCTTTT TTGTGCGTGC TCCACATTGG  
AAAATATAAT GACTGGGAAC AACGCGAAAA AACACGCACG AGGTGTAACC

28501 CTGCGGTTTC TCACATCGAA GTAGACTGCA TTCCAGCCTT CACAGTCTAT  
GACGCCAAAG AGTGTAGCTT CATCTGACGT AAGGTCGGA GTGTCAGATA

28551 TTGCTTTACG GATTTGTAC CCTCACGCTC ATCTGCAGCC TCATCACTGT  
AACGAAATGC CTAAACAGTG GGAGTGCGAG TAGACGTCGG AGTAGTGACA

28601 GGTCATCGCC TTTATCCAGT GCATTGACTG GGTCTGTGTG CGCTTTGCAT  
CCAGTAGCGG AAATAGGTCA CGTAACTGAC CCAGACACAC GCGAAACGTA

28651 ATCTCAGACA CCATCCCCAG TACAGGGACA GGACTATAGC TGAGCTTCTT  
TAGAGTCTGT GGTAGGGGTC ATGTCCCTGT CCTGATATCG ACTCGAAGAA

28701 AGAATTCTTT AATTATGAAA TTTACTGTGA CTTTTCTGCT GATTATTTGC  
TCTTAAGAAA TTAATACTTT AAATGACACT GAAAAGACGA CTAATAAACG

28751 ACCCTATCTG CGTTTTGTTC CCCGACCTCC AAGCCTCAAA GACATATATC  
TGGGATAGAC GCAAAACAAG GGGCTGGAGG TTCGGAGTTT CTGTATATAG

28801 ATGCAGATTC ACTCGTATAT GGAATATTCC AAGTTGCTAC AATGAAAAA  
TACGTCTAAG TGAGCATATA CTTATAAGG TTCAACGATG TTACTTTTTT

28851 GCGATCTTTC CGAAGCCTGG TTATATGCAA TCATCTCTGT TATGGTGTTC  
CGCTAGAAAG GCTTCGGACC AATATACGTT AGTAGAGACA ATACCACAAG

28901 TGCAGTACCA TCTTAGCCCT AGCTATATAT CCCTACCTTG ACATTGGCTG  
ACGTCATGGT AGAATCGGGA TCGATATATA GGGATGGAAC TGTAACCGAC

28951 GAACGCAATA GATGCCATGA ACCACCCAAC TTTCCCCGCG CCCGCTATGC  
CTTGCGTTAT CTACGGTACT TGGTGGGTTG AAAGGGGCGC GGGCGATACG

29001 TTCCACTGCA ACAAGTTGTT GCCGGCGGCT TTGTCCCAGC CAATCAGCCT  
AAGGTGACGT TGTTCAACAA CGGCCGCCGA AACAGGGTCG GTTAGTCGGA

29051 CGCCACCTT CTCCCACCCC CACTGAAATC AGCTACTTTA ATCTAACAGG  
GCGGGTGGAA GAGGGTGGGG GTGACTTTAG TCGATGAAAT TAGATTGTCC

29101 AGGAGATGAC TGACACCCTA GATCTAGAAA TGGACGGAAT TATTACAGAG  
TCCTCTACTG ACTGTGGGAT CTAGATCTTT ACCTGCCTTA ATAATGTCTC

29151 CAGCGCCTGC TAGAAAGACG CAGGGCAGCG GCCGAGCAAC AGCGCATGAA  
GTCGCGGACG ATCTTTCTGC GTCCCGTCGC CGGCTCGTTG TCGCGTACTT

29201 TCAAGAGCTC CAAGACATGG TTAACCTGCA CCAAGTCAAA AGGGGTATCT  
AGTTCTCGAG GTTCTGTACC AATTGAACGT GGTCACGTTT TCCCCATAGA

29251 TTTGTCTCGT AAAGCAGGCC AAAGTCACCT ACGACAGTAA TACCACCGGA  
AAACAGAGCA TTTCGTCCGG TTTCAGTGA TGCTGTCATT ATGGTGGCCT

29301 CACCGCCTTA GCTACAAGTT GCCAACCAAG CGTCAGAAAT TGGTGGTCAT  
GTGGCGGAAT CGATGTTCAA CGGTTGGTTC GCAGTCTTTA ACCACCAGTA

Figure 27 A E

29401 GCTGCATTCA CTCACCTTGT CAAGGACCTG AGGATCTCTG CACCCTTATT  
CGACGTAAGT GAGTGAACA GTTCCTGGAC TCCTAGAGAC GTGGGAATAA

29451 AAGACCCTGT GCGGTCTCAA AGATCTTATT CCCTTTAACT AATAAAAAAA  
TTCTGGGACA CGCCAGAGTT TCTAGAATAA GGGAAATTGA TTATTTTTTT

29501 AATAATAAAG CATCACTTAC TTAAATCAG TTAGCAAATT TCTGTCCAGT  
TTATTATTTT TAGTGAAATG AATTTTAGTC AATCGTTTAA AGACAGGTCA

29551 TTATTTCAGCA GCACCTCCTT GCCCTCCTCC CAGCTCTGGT ATTGCAGCTT  
AATAAGTCGT CGTGGAGGAA CGGGAGGAGG GTCGAGACCA TAACGTCGAA

29601 CCTCCTGGCT GCAAACCTTC TCCACAATCT AAATGGAATG TCAGTTTCCT  
GGAGGACCGA CGTTTGAAAG AGGTGTTAGA TTTACCTTAC AGTCAAAGGA

29651 CCTGTTCTTG TCCATCCGCA CCCACTATCT TCATGTTGTT GCAGATGAAG  
GGACAAGGAC AGGTAGGCGT GGGTGATAGA AGTACAACAA CGTCTACTTC

29701 CGCGCAAGAC CGTCTGAAGA TACCTTCAAC CCCGTGTATC CATATGACAC  
GCGCGTCTTG GCAGACTTCT ATGGAAGTTG GGGCACATAG GTATACTGTG

29751 GGAAACCGGT CCTCCAAC TGCCCTTTCT TACTCCTCCC TTTGTATCCC  
CCTTTGGCCA GGAGGTTGAC ACGGAAAAGA ATGAGGAGGG AAACATAGGG

29801 CCAATGGGTT TCAAGAGAGT CCCCCTGGGG TACTCTCTTT GCGCCTATCC  
GGTTACCCAA AGTTCTCTCA GGGGGACCCC ATGAGAGAAA CGCGGATAGG

29851 GAACCTCTAG TTACCTCCAA TGGCATGCTT GCGCTCAAAA TGGGCAACGG  
CTTGAGATC AATGGAGGTT ACCGTACGAA CGCGAGTTT ACCCGTTGCC

29901 CCTCTCTCTG GACGAGGCCG GCAACCTTAC CTCCCAAAT GTAACCACTG  
GGAGAGAGAC CTGCTCCGGC CGTTGGAATG GAGGGTTTA CATTGGTGAC

29951 TGAGCCCACC TCTCAAAAAA ACCAAGTCAA ACATAAACCT GGAAATATCT  
ACTCGGGTGG AGAGTTTTTT TGGTTCAGTT TGTATTGGA CTTTTATAGA

30001 GCACCCCTCA CAGTTACCTC AGAAGCCCTA ACTGTGGCTG CCGCCGCACC  
CGTGGGGAGT GTCAATGGAG TCTTCGGGAT TGACACCGAC GCGGCGTG

30051 TCTAATGGTC GCGGGCAACA CACTACCAT GCAATCACAG GCCCCGCTAA  
AGATTACCAG CGCCCGTTGT GTGAGTGGTA CGTTAGTGTC CGGGGCGATT

30101 CCGTGACGCA CTCCAACTT AGCATTGCCA CCCAAGGACC CCTCACAGTG  
GGCACGTGCT GAGGTTTGAA TCGTAACGGT GGGTTCCTGG GGAGTGTCAC

30151 TCAGAAGGAA AGCTAGCCCT GCAAACATCA GGCCCCCTCA CCACCACCGA  
AGTCTTCCTT TCGATCGGGA CGTTGTAGT CCGGGGGAGT GGTGGTGGCT

30201 TAGCAGTACC CTTACTATCA CTGCCTCACC CCCTCTAACT ACTGCCACTG  
ATCGTCATGG GAATGATAGT GACGGAGTGG GGGAGATTGA TGACGGTGAC

30251 GTAGCTTGGG CATTGACTTG AAAGAGCCCA TTTATACACA AAATGGAAAA  
CATCGAACCC GTAACCTGAAC TTTCTCGGGT AAATATGTGT TTTACCTTTT

Figure 27 AF

30351 TTTGACCGTA GCAACTGGTC CAGGTGTGAC TATTAATAAT ACTTCCTTGC  
AAACTGGCAT CGTTGACCAG GTCCACACTG ATAATTATTA TGAAGGAACG

30401 AAACATAAGT TACTGGAGCC TTGGGTTTTG ATTCACAAGG CAATATGCAA  
TTTGATTTCA ATGACCTCGG AACCCAAAAC TAAGTGTTCC GTTATACGTT

30451 CTTAATGTAG CAGGAGGACT AAGGATTGAT TCTCAAAACA GACGCCTTAT  
GAATTACATC GTCCTCCTGA TTCCTAACTA AGAGTTTTGT CTGCGGAATA

30501 ACTTGATGTT AGTTATCCGT TTGATGCTCA AAACCAACTA AATCTAAGAC  
TGAACTACAA TCAATAGGCA AACTACGAGT TTTGGTTGAT TTAGATTCTG

30551 TAGGACAGGG CCCTCTTTTT ATAACTCAG CCCACAACCT GGATATTAAAC  
ATCCTGTCCC GGGAGAAAAA TATTGAGTC GGGTGTTGAA CCTATAATTG

30601 TACAACAAAG GCCTTTACTT GTTTACAGCT TCAAACAATT CCAAAAAGCT  
ATGTTGTTTC CGGAAATGAA CAAATGTCGA AGTTTGTTAA GGTTTTTCGA

30651 TGAGGTTAAC CTAAGCACTG CCAAGGGGTT GATGTTTGAC GCTACAGCCA  
ACTCCAATTG GATTCGTGAC GGTTCCTCAA CTACAACTG CGATGTCGGT

30701 TAGCCATTAA TGCAGGAGAT GGGCTTGAAT TTGGTTCACC TAATGCACCA  
ATCGGTAATT ACGTCCTCTA CCCGAACTTA AACCAAGTGG ATTACGTGGT

30751 AACACAAATC CCCTCAAAAC AAAAATTGGC CATGGCCTAG AATTTGATTG  
TTGTGTTTAG GGGAGTTTTG TTTTAAACCG GTACCGGATC TTAAACTAAG

30801 AAACAAGGCT ATGGTTCCTA AACTAGGAAC TGGCCTTAGT TTTGACAGCA  
TTGTTCCTGA TACCAAGGAT TTGATCCTTG ACCGGAATCA AAACGTGCTG

30851 CAGGTGCCAT TACAGTAGGA AACAAAAATA ATGATAAGCT AACTTTGTGG  
GTCCACGGTA ATGTCATCCT TTGTTTTTAT TACTATTCTG TTGAAACACC

30901 ACCACACCAG CTCCATCTCC TAACTGTAGA CTAAATGCAG AGAAAGATGC  
TGGTGTGGTC GAGGTAGAGG ATTGACATCT GATTTACGTC TCTTTCTACG

30951 TAAACTCACT TTGGTCTTAA CAAAATGTGG CAGTCAAATA CTTGCTACAG  
ATTTGAGTGA AACCAGAATT GTTTTACACC GTCAGTTTAT GAACGATGTC

31001 TTTTCAGTTT GGCTGTAA AAGCAGTTTG CTCCAATATC TGGAACAGTT  
AAAGTCAAAA CCGACAATTT CCGTCAAACC GAGGTTATAG ACCTTGTCAA

31051 CAAAGTGCTC ATCTTATTAT AAGATTTGAC GAAAATGGAG TGCTACTAAA  
GTTTCACGAG TAGAATAATA TTCTAACTG CTTTACCTC ACGATGATTT

31101 CAATTCCTTC CTGGACCCAG AATATTGGAA CTTTAGAAAT GGAGATCTTA  
GTTAAGGAAG GACCTGGGTC TTATAACCTT GAAATCTTTA CCTCTAGAAT

31151 CTGAAGGCAC AGCCTATACA AACGCTGTTG GATTTATGCC TAACCTATCA  
GACTTCCGTG TCGGATATGT TTGCGACAAC CTAAATACGG ATTGGATAGT

31201 GCTTATCCAA AATCTCACGG TAAACTGCC AAAAGTAACA TTGTCAGTCA  
CGAATAGGTT TTAGAGTGCC ATTTTGACGG TTTTCATTGT AACAGTCAGT

Figure 27 AG



31251 AGTTTACTTA AACGGAGACA AAACATAACC TGTAACACTA ACCATTACAC  
TCAAATGAAT TTGCCTCTGT TTTGATTGG ACATTGTGAT TGGTAATGTG

31301 TAAACGGTAC ACAGGAAACA GGAGACACAA CTCCAAGTGC ATACTCTATG  
ATTTGCCATG TGTCCTTTGT CCTCTGTGTT GAGGTCACG TATGAGATAC

31351 TCATTTTCAT GGGACTGGTC TGGCCACAAC TACATTAATG AAATATTTGC  
AGTAAAAGTA CCCTGACCAG ACCGGTGTG ATGTAATTAC TTTATAAACG

31401 CACATCCTCT TACACTTTTT CATACTTGC CCAAGAATAA AGAATCGTTT  
GTGTAGGAGA ATGTGAAAAA GTATGTAACG GGTCTTATT TCTTAGCAAA

31451 GTGTTATGTT TCAACGTGTT TATTTTCAA TTGCAGAAAA TTTCAAGTCA  
CACAATACAA AGTTGCACAA ATAAAAAGTT AACGTCTTTT AAAGTTCAGT

31501 TTTTTCATTC AGTAGTATAG CCCCACCACC ACATAGCTTA TACAGATCAC  
AAAAAGTAAG TCATCATATC GGGGTGGTGG TGTATCGAAT ATGTCTAGTG

31551 CGTACCTTAA TCAAACTCAC AGAACCTAG TATTCAACCT GCCACCTCCC  
GCATGGAATT AGTTTGAGTG TCTTGGGATC ATAAGTTGGA CGGTGGAGGG

31601 TCCCAACACA CAGAGTACAC AGTCCTTTCT CCCCGGCTGG CCTTAAAAAG  
AGGGTTGTGT GTCTCATGTG TCAGGAAAGA GGGGCCGACC GGAATTTTTC

31651 CATCATATCA TGGGTAACAG ACATATTCTT AGGTGTTATA TTCCACACGG  
GTAGTATAGT ACCCATGTG TGTATAAGAA TCCACAATAT AAGGTGTGCC

31701 TTTCTGTGCG AGCCAAACGC TCATCAGTGA TATTAATAAA CTCCCCGGGC  
AAAGGACAGC TCGGTTTGCG AGTAGTCACT ATAATTATTT GAGGGGCCCG

31751 AGCTCACTTA AGTTCATGTC GCTGTCCAGC TGCTGAGCCA CAGGCTGCTG  
TCGAGTGAAT TCAAGTACAG CGACAGGTCG ACGACTCGGT GTCCGACGAC

31801 TCCAACTTGC GGTGCTTAA CGGGCGGCGA AGGAGAAAGTC CACGCCTACA  
AGGTGGAACG CCAACGAATT GCCCGCGCT TCCTCTTCAG GTGCGGATGT

31851 TGGGGGTAGA GTCATAATCG TGCATCAGGA TAGGGCGGTG GTGCTGCAGC  
ACCCCCATCT CAGTATTAGC ACGTAGTCCT ATCCCGCCAC CACGACGTG

31901 AGCGCGCGAA TAACTGCTG CCGCGCCGC TCCGTCCTGC AGGAATACAA  
TCGCGCGCTT ATTTGACGAC GCGCGCGCG AGGCAGGACG TCCTTATGTT

31951 CATGGCAGTG GTCTCCTCAG CGATGATTG CACCGCCGC AGCATAAGGC  
GTACCGTCAC CAGAGGAGT GCTACTAAGC GTGGCGGGCG TCGTATTCCG

32001 GCCTTGTCCT CCGGGCACAG CAGCGACCC TGATCTCACT TAAATCAGCA  
CGGAACAGGA GGCCCGTGTG GTCGCGTGGG ACTAGAGTGA ATTTAGTCGT

32051 CAGTAACTGC AGCACAGCAC CACAATATTG TTCAAAATCC CACAGTGCAA  
GTCATTGACG TCGTGTGCTG GTGTTATAAC AAGTTTTAGG GTGTCACGTT

32101 GCGCTGTAT CCAAAGCTCA TGGCGGGGAC CACAGAACCC ACGTGGCCAT  
CCGCGACATA GGTTCGAGT ACCGCCCCTG GTGTCTTGGG TGCACCGGTA

32151 CATACCACAA GCGCAGGTAG ATTAAGTGGC GACCCCTCAT AAACACGCTG  
GTATGGTGTG CGCGTCCATC TAATTCACCG CTGGGGAGTA TTTGTGCGAC

Figure 27AH

32251 CCATATAAAC CTCTGATTAA ACATGGCGCC ATCCACCACC ATCCTAAACC  
GGTATATTTG GAGACTAATT TGTACCGCGG TAGGTGGTGG TAGGATTTGG

32301 AGCTGGCCAA AACCTGCCCC CCGGCTATAC ACTGCAGGGA ACCGGGACTG  
TCGACCGGTT TTGGACGGGC GGCCGATATG TGACGTCCCT TGGCCCTGAC

32351 GAACAATGAC AGTGGAGAGC CCAGGACTCG TAACCATGGA TCATCATGCT  
CTTGTTACTG TCACCTCTCG GGTCTGAGC ATTGGTACCT AGTAGTACGA

32401 CGTCATGATA TCAATGTTGG CACAACACAG GCACACGTGC ATACACTTCC  
GCAGTACTAT AGTTACAACC GTGTTGTGTC CGTGTGCACG TATGTGAAGG

32451 TCAGGATTAC AAGCTCCTCC CGCGTTAGAA CCATATCCCA GGAACAACC  
AGTCCTAATG TTCGAGGAGG GCGCAATCTT GGTATAGGST CCCTTGTTGG

32501 CATTCCTGAA TCAGCGTAAA TCCCACACTG CAGGGAAGAC CTCGCACGTA  
GTAAGGACTT AGTCGCATT AGGGTGTGAC GTCCCTTCTG GAGCGTGCAT

32551 ACTCACGTTG TGCATTGTCA AAGTGTTACA TTCGGGCAGC AGCGGATGAT  
TGAGTGCAAC ACGTAACAGT TTCACAATGT AAGCCCGTCG TCGCCTACTA

32601 CCTCCAGTAT GGTAGCGCGG GTTCTGTCT CAAAAGGAGG TAGACGATCC  
GGAGGTCATA CCATCGCGCC CAAAGACAGA GTTTTCTCC ATCTGCTAGG

32651 CTACTGTACG GAGTGCGCCG AGACAACCGA GATCGTGTG GTCGTAGTGT  
GATGACATGC CTCACGCGGC TCTGTTGGCT CTAGCACAA CAGCATCACA

32701 CATGCCAAAT GGAACGCCGG ACGTAGTCAT ATTTCTGAA GCAAAACCAG  
GTACGGTTTA CCTTGCGGCC TGCATCAGTA TAAAGGACTT CGTTTTGGTC

32751 GTGCGGGCGT GACAAACAGA TCTGCGTCTC CGGTCTCGCC GCTTAGATCG  
CACGCCGCA CTGTTTGTCT AGACGCAGAG GCCAGAGCGG CGAATCTAGC

32801 CTCTGTGTAG TAGTTGTAGT ATATCCACTC TCTCAAAGCA TCCAGGCGCC  
GAGACACATC ATCAACATCA TATAGGTGAG AGAGTTTCGT AGGTCCGCGG

32851 CCCTGGCTTC GGGTTCATG TAAACTCCTT CATGCGCCGC TGCCCTGATA  
GGGACCGAAG CCCAAGATAC ATTTGAGGAA GTACGCGGCG ACGGGACTAT

32901 ACATCCACCA CCGCAGAATA AGCCACACCC AGCCAACCTA CACATTGTT  
TGTAGGTGGT GCGCTCTTAT TCGGTGTGGG TCGGTTGGAT GTGTAAGCAA

32951 CTGCGAGTCA CACACGGGAG GAGCGGGAAG AGCTGGAAGA ACCATGTTTT  
GACGCTCAGT GTGTGCCCTC CTCGCCCTC TCGACCTTCT TGGTACAAAA

33001 TTTTTTTATT CCAAAAGATT ATCCAAAACC TCAAAATGAA GATCTATTAA  
AAAAAATAA GGTTTTCTAA TAGGTTTTGG AGTTTTACTT CTAGATAATT

33051 GTGAACGCGC TCCCTCCCG TGGCGTGGTC AAACCTCTACA GCCAAAGAAC  
CACTTGCGCG AGGGGAGGCC ACCGCACCAG TTTGAGATGT CGGTTTCTTG

33101 AGATAATGGC ATTTGTAAGA TGTTGCACAA TGGCTTCCAA AAGGCAAACG  
TCTATTACCG TAAACATTCT ACAACGTGT ACCGAAGGT TTCCGTTTGC

Figure 27 AI

33201 CTCTATAAAC ATTCCAGCAC CTTCAACCAT GCCCAAATAA TTCTCATCTC  
GAGATATTTG TAAGGTCGTG GAAGTTGGTA CGGGTTTATT AAGAGTAGAG

33251 GCCACCTTCT CAATATATCT CTAAGCAAAT CCCGAATATT AAGTCGGGCC  
CGGTGGAAGA GTTATATAGA GATTCGTTTA GGGCTTATAA TTCAGGCCGG

33301 ATTGTAAAAA TCTGCTCCAG AGCGCCCTCC ACCTTCAGCC TCAAGCAGCG  
TAACATTTTT AGACGAGGTC TCGCGGGAGG TGAAGTCGG AGTTCGTCGC

33351 AATCATGATT GCAAAAATTC AGGTTCTCTCA CAGACCTGTA TAAGATTCAA  
TTAGTACTAA CGTTTTTAAG TCCAAGGAGT GTCTGGACAT ATTCTAAGTT

33401 AAGCGGAACA TTAACAAAAA TACCGCGATC CCGTAGGTCC CTTCGCAGGG  
TTCGCCCTGT AATTGTTTTT ATGGCGCTAG GGCATCCAGG GAAGCGTCCC

33451 CCAGCTGAAC ATAATCGTGC AGGTCTGCAC GGACCAGCGC GGCCACTTCC  
GGTCGACTTG TATTAGCACG TCCAGACGTG CCTGGTCGCG CCGGTGAAGG

33501 CCGCCAGGAA CCATGACAAA AGAACCACACA CTGATTATGA CACGCATACT  
GGCGGTCCCTT GGTACTGTTT TCTTGGGTGT GACTAATACT GTGCGTATGA

33551 CGGAGCTATG CTAACCAGCG TAGCCCCGAT GTAAGCTTGT TGCATGGGCG  
GCCTCGATAC GATTGGTCGC ATCGGGGCTA CATTCTGAACA ACGTACCCGC

33601 GCGATATAAA ATGCAAGGTG CTGCTCAAAA AATCAGGCAA AGCCTCGCGC  
CGCTATATTT TACGTCCAC GACGAGTTT TTAGTCCGTT TCGGAGCGCG

33651 AAAAAAGAAA GCACATCGTA GTCATGCTCA TGCAGATAAA GGCAGGTAAG  
TTTTTCTTT CGTGTAGCAT CAGTACGAGT ACGTCTATTT CCGTCCATTC

33701 CTCCGGAACC ACCACAGAAA AAGACACCAT TTTTCTCTCA AACATGTCTG  
GAGGCCTTGG TGGTGCTTTT TTCTGTGGTA AAAAGAGAGT TTGTACAGAC

33751 CGGGTTTCTG CATAAACACA AAATAAAATA ACAAAAAAAC ATTTAAACAT  
GCCCAAAGAC GTATTTGTGT TTTATTTTAT TGTTTTTTTG TAAATTTGTA

33801 TAGAAGCCTG TCTTACAACA GGAAAAACAA CCCTTATAAG CATAAGACGG  
ATCTTCGGAC AGAATGTTGT CCTTTTGTGTT GGAATATTC GTATTCTGCC

33851 ACTACGGCCA TGCCGGCGTG ACCGTAAAAA AACTGGTCAC CGTGATTAAA  
TGATGCCGGT ACGGCCGCAC TGGCATTTTT TTGACCAGTG GCACATAATT

33901 AAGCACCACC GACAGCTCCT CGGTCATGTC CGGAGTCATA ATGTAAGACT  
TTCGTGGTGG CTGTCGAGGA GCCAGTACAG GCCTCAGTAT TACATTCTGA

33951 CGGTAAACAC ATCAGGTTGA TTCACATCGG TCAGTGCTAA AAAGCGACCG  
GCCATTTGTG TAGTCCAAC TAAAGTAGCC AGTCACGATT TTTCGCTGGC

34001 AAATAGCCCG GGGGAATACA TACCCGCAGG CGTAGAGACA ACATTACAGC  
TTTATCGGGC CCCCTTATGT ATGGGCGTCC GCATCTCTGT TGTAAATGTCG

34051 CCCCATAGGA GGTATAACAA AATTAATAGG AGAGAAAAAC ACATAAACAC  
GGGGTATCCT CCATATTGTT TTAATTATCC TCTCTTTTGG TGTATTGTG

Figure 27A5

34151 ACATACAGCG CTTCCACAGC GGCAGCCATA ACAGTCAGCC TTACCAGTAA  
 TGTATGTCGC GAAGGTGTCG CCGTCGGTAT TGTGAGTCGG AATGGTCATT  
 34201 AAAAGAAAAC CTATTAAAAA AACACCACTC GACACGGCAC CAGCTCAATC  
 TTTTCCTTTG GATAATTTTT TTGTGGTGAG CTGTGCCGTG GTCGAGTTAG  
 34251 AGTCACAGTG TAAAAAAGGG CCAAGTGCAG AGCGAGTATA TATAGGACTA  
 TCAGTGTAC ATTTTTTCCC GGTTACGTC TCGTCATAT ATATCCTGAT  
 34301 AAAAATGACG TAACGGTTAA AGTCCACAAA AAACACCCAG AAAACCGCAC  
 TTTTACTGCT ATTGCCAATT TCAGGTGTTT TTTGTGGGTC TTTTGGCGTG  
 34351 GCGAACCTAC GCCCAGAAAC GAAAGCCAAA AAACCCACAA CTTCTCTAAA  
 CGCTTGATG CGGGTCTTTG CTTTCGGTTT TTTGGGTGTT GAAGGAGTTT  
 34401 TCGTCACTTC CGTTTTCCCA CGTTACGTCA CTTCCCATTT TAAGAAAAC  
 AGCAGTGAAG GCAAAAGGGT GCAATGCAGT GAAGGGTAAA ATTCTTTTGA  
 34451 ACAATTCCCA ACACATACAA GTTACTCCGC CCTAAAACCT ACGTCACCCG  
 TGTAAAGGGT TGTGTATGTT CAATGAGGCG GGATTTTGA TGCAGTGGGC  
 34501 CCCCCTTCCC ACGCCCCGCG CCACGTCACA AACTCCACCC CCTCATTATC  
 GGGGCAAGGG TCGGGGGCGC GGTGCAGTGT TTGAGGTGGG GGAGTAATAG  
 PacI  
 -----  
 34551 ATATTGGCTT CAATCCAAAA TAAGGTATAT TATTGATGAT GTTAATTAAAG  
 TATAACCGAA GTTAGGTTTT ATTCCATATA ATAACCTACTA CAATTAATTC  
 34601 AATTCGGATC TGCGACGCGA GGCTGGATGG CCTTCCCCAT TATGATTCTT  
 TTAAGCCTAG ACGCTGCGCT CCGACCTACC GGAAGGGGTA ATACTAAGAA  
 34651 CTCGCTTCCG GCGGCATCGG GATGCCCGCG TTGCAGGCCA TGCTGTCCAG  
 GAGCGAAGGC CGCCGTAGCC CTACGGGCGC AACGTCCGGT ACGACAGGTC  
 34701 GCAGGTAGAT GACGACCATC AGGGACAGCT TCAAGGCCAG CAAAAGGCCA  
 CGTCCATCTA CTGCTGGTAG TCCCTGTCTGA AGTTCCGGTC GTTTTCCGGT  
 34751 GGAACCGTAA AAAGGCCGCG TTGCTGGCGT TTTTCCATAG GCTCCGCCCC  
 CCTTGGCATT TTTCCGGCGC AACGACCGCA AAAAGGTATC CGAGGCGGGG  
 34801 CCTGACGAGC ATCACAAAAA TCGACGCTCA AGTCAGAGGT GGCAGAACCC  
 GGACTGCTCG TAGTGTTTTT AGCTGCGAGT TCAGTCTCCA CCGCTTTGGG  
 34851 GACAGGACTA TAAAGATACC AGGCGTTTCC CCCTGGAAGC TCCCTCGTGC  
 CTGTCCTGAT ATTTCTATGG TCCGCAAAGG GGGACCTTCG AGGGAGCAGC  
 34901 GCTCTCCTGT TCCGACCCTG CCGCTTACCG GATACCTGTC CGCCTTTCTC  
 CGAGAGGACA AGGCTGGGAC GGCGAATGGC CTATGGACAG GCGGAAAGAG  
 34951 CCTTCGGGAA GCGTGGCGCT TTCTCATAGC TCACGCTGTA GGTATCTCAG  
 GGAAGCCCTT CGCACC CGA AAGAGTATCG AGTGCACAT CCATAGAGTC  
 35001 TTCGGTGTAG GTCGTTGCT CCAAGCTGGG CTGTGTGCAC GAACCCCCCG  
 AAGCCACATC CAGCAAGCGA GGTTCGACCC GACACACGTG CTTGGGGGGC

Figure 27 AK

AAGTCGGGCT GGCACGCGG AATAGGCCAT TGATAGCAGA ACTCAGGTTG

35101 CCGGTAAGAC ACGACTTATC GCCACTGGCA GCAGCCACTG GTAACAGGAT  
GGCCATTCTG TGCTGAATAG CGGTGACCGT CGTCGGTGAC CATTGTCCTA

35151 TAGCAGAGCG AGGTATGTAG GCGGTGCTAC AGAGTTCTTG AAGTGGTGGC  
ATCGTCTCGC TCCATACATC CGCCACGATG TCTCAAGAAC TTCACCACCG

35201 CTAACCTACGG CTACACTAGA AGGACAGTAT TTGGTATCTG CGCTCTGCTG  
GATTGATGCC GATGTGATCT TCCTGTCATA AACCATAGAC GCGAGACGAC

35251 AAGCCAGTTA CCTTCGGAAA AAGAGTTGGT AGCTCTTGAT CCGGCAAACA  
TTCGGTCAAT GGAAGCCTTT TTCTCAACCA TCGAGAACTA GGCCGTTTGT

35301 AACCACCGCT GGTAGCGGTG GTTTTTTGT TTGCAAGCAG CAGATTACGC  
TTGGTGGCGA CCATCGCCAC CAAAAAACA AACGTTCGTC GTCTAATGCG

35351 GCAGAAAAAA AGGATCTCAA GAAGATCCTT TGATCTTTTC TACGGGGTCT  
CGTCTTTTTT TCCTAGAGTT CTCTAGGAA ACTAGAAAAG ATGCCCCAGA

35401 GACGCTCAGT GGAACGAAAA CTCACGTAA GGGATTTTGG TCATGAGATT  
CTGCGAGTCA CCTTGCTTTT GAGTGCAATT CCCTAAAACC AGTACTCTAA

35451 ATCAAAAAGG ATCTTCACCT AGATCCTTTT AAATCAATCT AAAGTATATA  
TAGTTTTTCC TAGAAGTGGA TCTAGGAAA TTTAGTTAGA TTTCATATAT

35501 TGAGTAACT TGGTCTGACA GTTACCAATG CTTAATCAGT GAGGCACCTA  
ACTCATTGTA ACCAGACTGT CAATGGTTAC GAATTAGTCA CTCCGTGGAT

35551 TCTCAGCGAT CTGTCTATTT CGTTCATCCA TAGTTGCCTG ACTCCCCGTC  
AGAGTCGCTA GACAGATAAA GCAAGTAGGT ATCAACGGAC TGAGGGGCAG

35601 GTGTAGATAA CTACGATACG GGAGGGCTTA CCATCTGGCC CCAGTGCTGC  
CACATCTATT GATGCTATGC CCTCCGAAT GGTAGACCGG GGTCACGACG

35651 AATGATACCG CGAGACCCAC GCTCACCAGC TCCAGATTTA TCAGCAATAA  
TTACTATGGC GCTCTGGGTG CGAGTGCCG AGGTCTAAAT AGTCGTTATT

35701 ACCAGCCAGC CGGAAGGGCC GAGCGCAGAA GTGGTCCTGC AACTTTATCC  
TGGTCGGTCG GCCTTCCCGG CTCGCGTCTT CACCAGGACG TTGAAATAGG

35751 GCCTCCATCC AGTCTATTAA TTGTTGCCGG GAAGCTAGAG TAAGTAGTTC  
CGGAGGTAGG TCAGATAATT AACAACGGCC CTTGATCTC ATTCAATCAAG

35801 GCCAGTTAAT AGTTTGCGCA ACGTTGTTGC CATTGCTACA GGCATCGTGG  
CGGTCAATTA TCAAACGCGT TGCAACAACG GTAACGATGT CCGTAGCACC

35851 TGTCACGCTC GTCGTTTGGT ATGGCTTCAT TCAGCTCCGG TTCCCAACGA  
ACAGTGCGAG CAGCAAACCA TACCGAAGTA AGTCGAGGCC AAGGGTTGCT

35901 TCAAGGCGAG TTACATGATC CCCCATGTTG TGCAAAAAAG CGGTTAGCTC  
AGTTCCGCTC AATGTACTAG GGGGTACAAC ACGTTTTTTC GCCAATCGAG

35951 CTTCCGTCCT CCGATCGTTG TCAGAAGTAA GTTGGCCGCA GTGTTATCAC  
GAAGCCAGGA GGCTAGCAAC AGTCTTCATT CAACCGCGT CACAATAGTG

Figure 2 AL

36051 AGATGCTTTT CTGTGACTGG TGAGTACTCA ACCAAGTCAT TCTGAGAATA  
TCTACGAAAA GACACTGACC ACTCATGAGT TGGTTCAGTA AGACTCTTAT

36101 GTGTATGCGG CGACCGAGTT GCTCTTGCCC GCGGTCAACA CGGGATAATA  
CACATACGCC GCTGGCTCAA CGAGAACGGG CCGCAGTTGT GCCCTATTAT

36151 CCGCGCCACA TAGCAGAACT TTAAGAGTGC TCATCATTGG AAAACGTTCT  
GGCGCGGTGT ATCGTCTTGA AATTTTCACG AGTAGTAACC TTTTGCAAGA

36201 TCGGGGCGAA AACTCTCAAG GATCTTACCG CTGTTGAGAT CCAGTTCGAT  
AGCCCCGCTT TTGAGAGTTC CTAGAATGGC GACAACTCTA GGTCAGCTA

36251 GTAACCCACT CGTGCACCCA ACTGATCTTC AGCATCTTTT ACTTTCACCA  
CATTGGGTGA GCACGTGGGT TGACTAGAAG TCGTAGAAAA TGAAAGTGGT

36301 GCGTTTCTGG GTGAGCAAAA ACAGGAAGGC AAAATGCCGC AAAAAAGGGA  
CGCAAAGACC CACTCGTTTT TGTCTTCCG TTTTACGGCG TTTTTCCTT

36351 ATAAGGGCGA CACGGAAATG TTGAATACTC ATACTCTTCC TTTTCAATA  
TATTCCCGCT GTGCCTTTAC AACTTATGAG TATGAGAAGG AAAAAGTTAT

36401 TTATTGAAGC ATTTATCAGG GTTATTGTCT CATGAGCGGA TACATATTTG  
AATAACTTCG TAAATAGTCC CAATAACAGA GTACTCGCCT ATGTATAAAC

36451 AATGTATTTA GAAAAATAAA CAAATAGGGG TTCCGCGCAC ATTTCCCCGA  
TTACATAAAT CTTTTTATTT GTTTATCCCC AAGGCGCGTG TAAAGGGGCT

36501 AAAGTGCCAC CTGACGTCTA AGAAACCATT ATTATCATGA CATTAACCTA  
TTTCACGGTG GACTGCAGAT TCTTTGGTAA TAATAGTACT GTAATTGGAT

36551 TAAAAATAGG CGTATCACGA GGCCCTTTTCG TCTTCAAGAA TTGGATCCGA  
ATTTTTATCC GCATAGTGCT CCGGGAAAGC AGAAGTTCTT AACCTAGGCT

PacI

36601 ATTCTTAATT TCTTAATTAA (SEQ ID NO:34)  
TAAGAATTAA AGAATTAATT (SEQ ID NO:35)

Figure 27AM

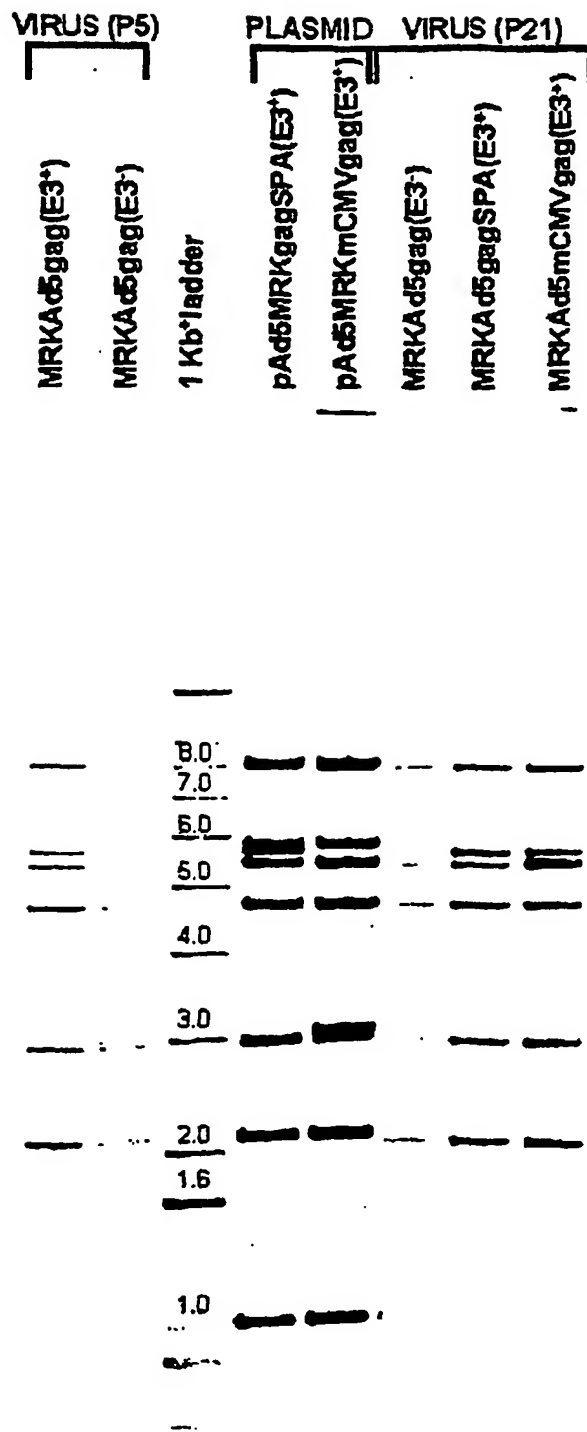


FIGURE 28

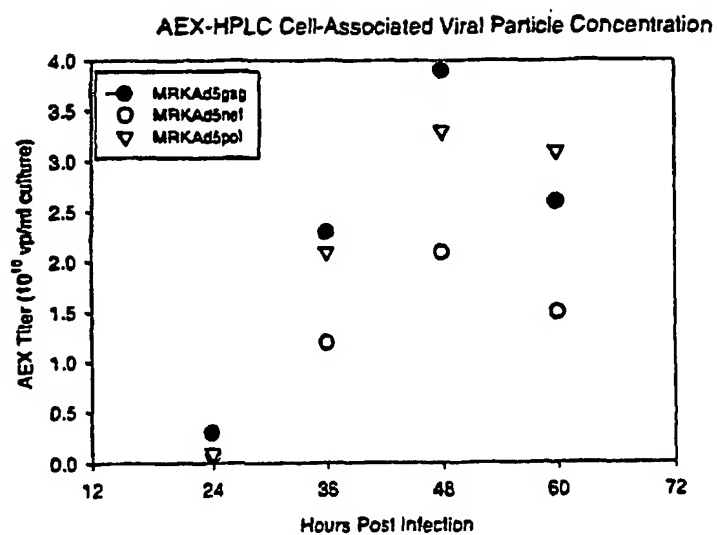


FIGURE 29A

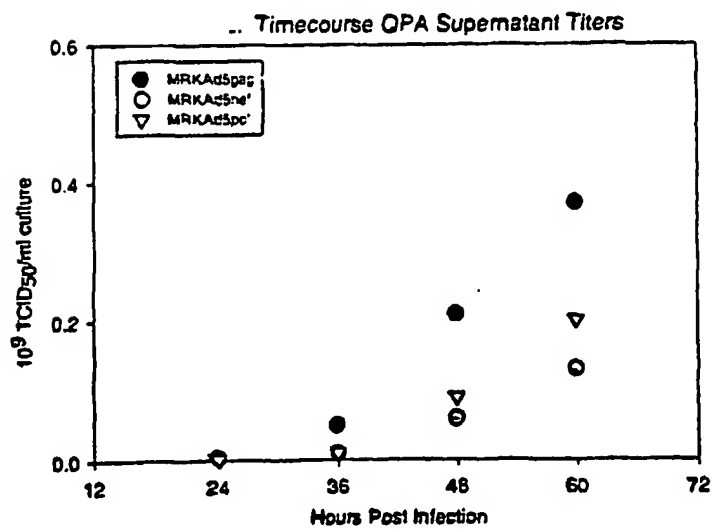


FIGURE 29B



atg gat gca atg aag aga ggg ctc tgc tgt gtg ctg ctg ctg tgt gga Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly 1 5 10 15	48
gca gtc ttc gtt tgc ccc agc gag atc tcc att gtg tgg gcc tcc agg Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ile Val Trp Ala Ser Arg 20 25 30	96
gag ctg gag agg ttt gct gtg aac cct ggc ctg ctg gag acc tct gag Glu Leu Glu Arg Phe Ala Val Asn Pro Gly Leu Leu Glu Thr Ser Glu 35 40 45	144
ggg tgc agg cag atc ctg ggc cag ctc cag ccc tcc ctg caa aca ggc Gly Cys Arg Gln Ile Leu Gly Gln Leu Gln Pro Ser Leu Gln Thr Gly 50 55 60	192
tct gag gag ctg agg tcc ctg tac aac aca gtg gct acc ctg tac tgt Ser Glu Glu Leu Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys 65 70 75 80	240
gtg cac cag aag att gat gtg aag gac acc aag gag gcc ctg gag aag Val His Gln Lys Ile Asp Val Lys Asp Thr Lys Glu Ala Leu Glu Lys 85 90 95	288
att gag gag gag cag aac aag tcc aag aag aag gcc cag cag gct gct Ile Glu Glu Glu Gln Asn Lys Ser Lys Lys Lys Ala Gln Gln Ala Ala 100 105 110	336
gct ggc aca ggc aac tcc agc cag gtg tcc cag aac tac ccc att gtg Ala Gly Thr Gly Asn Ser Ser Gln Val Ser Gln Asn Tyr Pro Ile Val 115 120 125	384
cag aac ctc cag ggc cag atg gtg cac cag gcc atc tcc ccc cgg acc Gln Asn Leu Gln Gly Gln Met Val His Gln Ala Ile Ser Pro Arg Thr 130 135 140	432
ctg aat gcc tgg gtg aag gtg gtg gag gag aag gcc ttc tcc cct gag Leu Asn Ala Trp Val Lys Val Val Glu Glu Lys Ala Phe Ser Pro Glu 145 150 155 160	480
gtg atc ccc atg ttc tct gcc ctg tct gag ggt gcc acc ccc cag gac Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala Thr Pro Gln Asp 165 170 175	528
ctg aac acc atg ctg aac aca gtg ggg ggc cat cag gct gcc atg cag Leu Asn Thr Met Leu Asn Thr Val Gly Gly His Gln Ala Ala Met Gln 180 185 190	576
atg ctg aag gag acc atc aat gag gag gct gct gag tgg gac agg ctg Met Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala Glu Trp Asp Arg Leu 195 200 205	624
cat cct gtg cac gct ggc ccc att gcc ccc ggc cag atg agg gag ccc His Pro Val His Ala Gly Pro Ile Ala Pro Gly Gln Met Arg Glu Pro 210 215 220	672
agg ggc tct gac att gct ggc acc acc tcc acc ctc cag gag cag att Arg Gly Ser Asp Ile Ala Gly Thr Thr Ser Thr Leu Gln Glu Gln Ile 225 230 235 240	720
ggc tgg atg acc aac aac ccc ccc atc cct gtg ggg gaa atc tac aag Gly Trp Met Thr Asn Asn Pro Pro Ile Pro Val Gly Glu Ile Tyr Lys 245 250 255	768

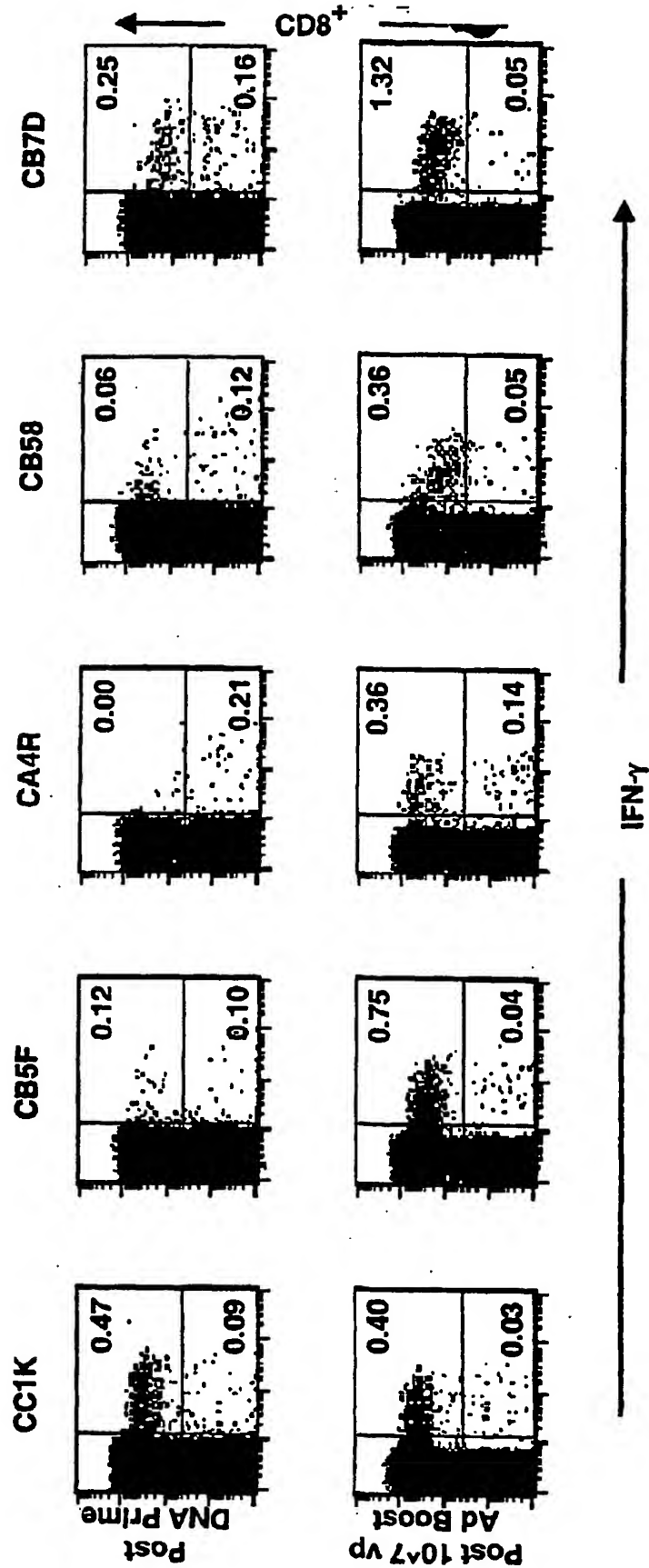
Figure 30A

agg tgg atc atc ctg ggc ctg aac aag att gtg agg atg tac tcc ccc Arg Trp Ile Ile Leu Gly Leu Asn Lys Ile Val Arg Met Tyr Ser Pro 260 265 270	816
acc tcc atc ctg gac atc agg cag ggc ccc aag gag ccc ttc agg gac Thr Ser Ile Leu Asp Ile Arg Gln Gly Pro Lys Glu Pro Phe Arg Asp 275 280 285	864
tat gtg gac agg ttc tac aag acc ctg agg gct gag cag gcc tcc cag Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala Glu Gln Ala Ser Gln 290 295 300	912
gag gtg aag aac tgg atg aca gag acc ctg ctg gtg cag aat gcc aac Glu Val Lys Asn Trp Met Thr Glu Thr Leu Val Gln Asn Ala Thr Asn 305 310 315 320	960
cct gac tgc aag acc atc ctg aag gcc ctg ggc cct gct gcc acc ctg Pro Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro Ala Ala Thr Leu 325 330 335	1008
gag gag atg atg aca gcc tgc cag ggg gtg ggg ggc cct ggt cac aag Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly Pro Gly His Lys 340 345 350	1056
gcc agg gtg ctg gct gag gcc atg tcc cag gtg acc aac tcc gcc acc Ala Arg Val Leu Ala Glu Ala Met Ser Gln Val Thr Asn Ser Ala Thr 355 360 365	1104
atc atg atg cag agg ggc aac ttc agg aac cag agg aag aca gtg aag Ile Met Met Gln Arg Gly Asn Phe Arg Asn Gln Arg Lys Thr Val Lys 370 375 380	1152
tgc ttc aac tgt ggc aag gtg ggc cac att gcc aag aac tgt agg gcc Cys Phe Asn Cys Gly Lys Val Gly His Ile Ala Lys Asn Cys Arg Ala 385 390 395 400	1200
ccc agg aag aag ggc tgc tgg aag tgt ggc aag gag ggc cac cag atg Pro Arg Lys Lys Gly Cys Trp Lys Cys Gly Lys Glu Gly His Gln Met 405 410 415	1248
aag gac tgc aat gag agg cag gcc aac ttc ctg ggc aaa atc tgg ccc Lys Asp Cys Asn Glu Arg Gln Ala Asn Phe Leu Gly Lys Ile Trp Pro 420 425 430	1296
tcc cac aag ggc agg cct ggc aac ttc ctc cag tcc agg cct gag ccc Ser His Lys Gly Arg Pro Gly Asn Phe Leu Gln Ser Arg Pro Glu Pro 435 440 445	1344
aca gcc cct ccc gag gag tcc ttc agg ttc ggg gag gag aag acc acc Thr Ala Pro Pro Glu Glu Ser Phe Arg Phe Gly Glu Glu Lys Thr Thr 450 455 460	1392
ccc agc cag aag cag gag ccc att gac aag gag ctg tac ccc ctg gcc Pro Ser Gln Lys Gln Glu Pro Ile Asp Lys Glu Leu Tyr Pro Leu Ala 465 470 475 480	1440
tcc ctg agg tcc ctg ttt ggc aac gac ccc tcc tcc cag taa (SID NO:36) Ser Leu Arg Ser Leu Phe Gly Asn Asp Pro Ser Ser Gln * (SID NO:37) 485 490	1482

Figure 30 B

**Figure 31**

**IFN- $\gamma$  Secretion against Gag 20-aa pool from CD3<sup>+</sup> T cells of Monkey PBMCs**



# Immunizations



FIGURE 33A

ATGGGTGCTA GGGCTTCTGT GCTGTCTGGT GGTGAGCTGG ACAAGTGGGA GAAGATCAGG  
 CTGAGGCCTG GTGGCAAGAA GAAGTACAAG CTAAAGCACA TTGTGTGGGC CTCCAGGGAG  
 CTGGAGAGGT TTGCTGTGAA CCCTGGCCTG CTGGAGACCT CTGAGGGGTG CAGGCAGATC  
 CTGGGCCAGC TCCAGCCCTC CCTGCAAACA GGCTCTGAGG AGCTGAGGTC CCTGTACAAC  
 ACAGTGGCTA CCCTGTACTG TGTGCACCAG AAGATTGATG TGAAGGACAC CAAGGAGGCC  
 CTGGAGAAGA TTGAGGAGGA GCAGAACAAAG TCCAAGAAGA AGGCCAGCA GGCTGCTGCT  
 GGCACAGGCA ACTCCAGCCA GGTGTCCCAG AACTACCCCA TTGTGCAGAA CCTCCAGGGC  
 CAGATGGTGC ACCAGGCCAT CTCCCCCGG ACCCTGAATG CCTGGGTGAA GGTGGTGGAG  
 GAGAAGGCCT TCTCCCTGA GGTGATCCCC ATGTTCTCTG CCCTGTCTGA GGGTGCCACC  
 CCCCAGGACC TGAACACCAT GCTGAACACA GTGGGGGGCC ATCAGGCTGC CATGCAGATG  
 CTGAAGGAGA CCATCAATGA GGAGGTGCT GAGTGGGACA GGCTGCATCC TGTGCACGCT  
 GGCCCCATTG CCCCCGGCCA GATGAGGGAG CCCAGGGGCT CTGACATTGC TGGCACCACC  
 TCCACCCTCC AGGAGCAGAT TGGCTGGATG ACCAACACC CCCCATCCC TGTGGGGGAA  
 ATCTACAAGA GGTGGATCAT CCTGGGCCTG AACAAGATTG TGAGGATGTA CTCCCCCACC  
 TCCATCCTGG ACATCAGGCA GGGCCCCAAG GAGCCCTTCA GGGACTATGT GGACAGGTTT  
 TACAAGACCC TGAGGGCTGA GCAGGCCTCC CAGGAGGTGA AGAACTGGAT GACAGAGACC  
 CTGCTGGTGC AGAATGCCAA CCCTGACTGC AAGACCATCC TGAAGGCCCT GGGCCCTGCT  
 GCCACCCTGG AGGAGATGAT GACAGCCTGC CAGGGGGTGG GGGGCCCTGG TCACAAGGCC  
 AGGGTGCTGG CTGAGGCCAT GTCCCAGGTG ACCAATCCG CCACCATCAT GATGCAGAGG  
 GGCAACTTCA GGAACCAGAG GAAGACAGTG AAGTGCTTCA ACTGTGGCAA GGTGGGCCAC  
 ATTGCCAAGA ACTGTAGGGC CCCCAGGAAG AAGGGCTGCT GGAAGTGTGG CAAGGAGGGC  
 CACCAGATGA AGGACTGCAA TGAGAGGCAG GCCAACTTCC TGGGCAAAAT CTGGCCCTCC  
 CACAAGGGCA GGCTTGCAA CTTCTCCAG TCCAGGCCTG AGCCACAGC CCCTCCCGAG  
 GAGTCCTTCA GGTGTGGGA GGAGAAGACC ACCCCCAGCC AGAAGCAGGA GCCCATTGAC  
 AAGGAGCTGT ACCCCCTGGC CTCCCTGAGG TCCCTGTTG GCAACGACCC CTCTCCCGAG  
 ATGGCTCCCA TCTCCCCCAT TGAGACTGTG CCTGTGAAGC TGAAGCCTGG CATGGATGGC  
 CCAAGGTGA AGCAGTGGCC CCTGACTGAG GAGAAGATCA AGGCCCTGGT GGAAATCTGC  
 ACTGAGATGG AGAAGGAGGG CAAAATCTCC AAGATTGGCC CCGAGAACCC CTACAACACC  
 CCTGTGTTTG CCATCAAGAA GAAGGACTCC ACCAAGTGA GGAAGCTGGT GGACTTCAGG  
 GAGCTGAACA AGAGGACCCA GGACTTCTGG GAGGTGCAGC TGGGCATCCC CCACCCCGCT  
 GGCTGAAGA AGAAGAAGTC TGTGACTGTG CTGGCTGTGG GGGATGCCTA CTTCTCTGTG  
 CCCCTGGATG AGGACTTCAG GAAGTACACT GCCTTCACCA TCCCCCTCAT CAACAATGAG  
 ACCCCTGGCA TCAGGTACCA GTACAATGTG CTGCCCCAGG GCTGGAAGGG CTCCCCTGCC  
 ATCTTCCAGT CCTCCATGAC CAAGATCCTG GAGCCCTTCA GGAAGCAGAA CCCTGACATT  
 GTGATCTACC AGTACATGGC TGCCCTGTAT GTGGGCTCTG ACCTGGAGAT TGGGCAGCAC  
 AGGACCAAGA TTGAGGAGCT GAGGCAGCAC CTGCTGAGGT GGGGCCTGAC CACCCCTGAC  
 AAGAAGCACC AGAAGGAGCC CCCCCTCCTG TGGATGGGCT ATGAGCTGCA CCCCAGACAAG  
 TGGACTGTGC AGCCCATTTG GCTGCCCTGAG AAGGACTCCT GGACTGTGAA TGACATCCAG  
 AAGCTGGTGG GCAAGCTGAA CTGGGCCTCC CAAATCTACC CTGGCATCAA GGTGAGGCAG  
 CTGTGCAAGC TGCTGAGGGG CACCAAGGCC CTGACTGAGG TGATCCCCCT GACTGAGGAG  
 GCTGAGCTGG AGCTGGCTGA GAACAGGGAG ATCCTGAAGG AGCCTGTGCA TGGGGTGTAC

FIGURE 33B

TATGACCCCT CCAAGGACCT GATTGCTGAG ATCCAGAAGC AGGGCCAGGG CCAGTGGACC  
TACCAAATCT ACCAGGAGCC CTTCAAGAAC CTGAAGACTG GCAAGTATGC CAGGATGAGG  
GGGGCCCA CCAATGATGT GAAGCAGCTG ACTGAGGCTG TGCAGAAGAT CACCACTGAG  
TCCATTGTGA TCTGGGGCAA GACCCCCAAG TTCAAGCTGC CCATCCAGAA GGAGACCTGG  
GAGACCTGGT GGA CTGAGTA CTGGCAGGCC ACCTGGATCC CTGAGTGGGA GTTTGTGAAC  
ACCCCCCCC TGGTGAAGCT GTGGTACCAG CTGGAGAAGG AGCCCATGTG GGGGGCTGAG  
ACCTTCTATG TGGCTGGGGC TGCCAACAGG GAGACCAAGC TGGGCAAGGC TGGCTATGTG  
ACCAACAGGG GCAGGCAGAA GGTGGTGACC CTGACTGACA CCACCAACCA GAAGACTGCC  
CTCCAGGCCA TCTACCTGGC CCTCCAGGAC TCTGGCCTGG AGGTGAACAT TGTGACTGCC  
TCCAGTATG CCCTGGGCAT CATCCAGGCC CAGCCTGATC AGTCTGAGTC TGAGCTGGTG  
AACCAGATCA TTGAGCAGCT GATCAAGAAG GAGAAGGTGT ACCTGGCCTG GGTGCTGCC  
CACAAGGGCA TTGGGGGCAA TGAGCAGGTG GACAAGCTGG TGTCTGCTGG CATCAGGAAG  
GTGCTGTTCC TGGATGGCAT TGACAAGGCC CAGGATGAGC ATGAGAAGTA CCACTCCAAC  
TGGAGGGCTA TGGCCTCTGA CTTCAACCTG CCCCCTGTGG TGGCTAAGGA GATTGTGGCC  
TCCTGTGACA AGTGCCAGCT GAAGGGGGAG GCCATGCATG GGCAGGTGGA CTGCTCCCCT  
GGCATCTGGC AGCTGGCCTG CACCCACCTG GAGGGCAAGG TGATCCTGGT GGCTGTGCAT  
GTGGCCTCCG GCTACATTGA GGCTGAGGTG ATCCCTGCTG AGACAGGCCA GGAGACTGCC  
TACTTCCTGC TGAAGCTGGC TGGCAGGTGG CCTGTGAAGA CCATCCACAC TGCCAATGGC  
TCCAACCTCA CTGGGGCCAC AGTGAGGGCT GCCTGCTGGT GGGCTGGCAT CAAGCAGGAG  
TTTGGCATCC CCTACAACCC CCAGTCCCAG GGGGTGGTGG CCTCCATGAA CAAGGAGCTG  
AAGAAGATCA TTGGGCAGGT GAGGGACCAG GCTGAGCACC TGAAGACAGC TGTGCAGATG  
GCTGTGTTCA TCCACAACCT CAAGAGGAAG GGGGGCATCG GGGGCTACTC CGCTGGGGAG  
AGGATTGTGG ACATCATTGC CACAGACATC CAGACCAAGG AGCTCCAGAA GCAGATCACC  
AAGATCCAGA ACTTCAGGGT GTACTACAGG GACTCCAGGA ACCCCCTGTG GAAGGGCCCT  
GCCAAGCTGC TGTGGAAGGG GGAGGGGGCT GTGGTGATCC AGGACAACCTC TGACATCAAG  
GTGGTGCCCA GGAGGAAGGC CAAGATCATC AGGGACTATG GCAAGCAGAT GGCTGGGGAT  
GACTGTGTGG CCTCCAGGCA GGATGAGGAC TAA

SEQ ID NO: 38

FIGURE 34A

Met Gly Ala Arg Ala Ser Val Leu Ser Gly Gly Glu Leu Asp Lys Trp Glu Lys  
 Ile Arg Leu Arg Pro Gly Gly Lys Lys Lys Tyr Lys Leu Lys His Ile Val Trp  
 Ala Ser Arg Glu Leu Glu Arg Phe Ala Val Asn Pro Gly Leu Leu Glu Thr Ser  
 Glu Gly Cys Arg Gln Ile Leu Gly Gln Leu Gln Pro Ser Leu Gln Thr Gly Ser  
 Glu Glu Leu Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys Val His Gln  
 Lys Ile Asp Val Lys Asp Thr Lys Glu Ala Leu Glu Lys Ile Glu Glu Glu Gln  
 Asn Lys Ser Lys Lys Lys Ala Gln Gln Ala Ala Ala Gly Thr Gly Asn Ser Ser  
 Gln Val Ser Gln Asn Tyr Pro Ile Val Gln Asn Leu Gln Gly Gln Met Val His  
 Gln Ala Ile Ser Pro Arg Thr Leu Asn Ala Trp Val Lys Val Val Glu Glu Lys  
 Ala Phe Ser Pro Glu Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala Thr  
 Pro Gln Asp Leu Asn Thr Met Leu Asn Thr Val Gly Gly His Gln Ala Ala Met  
 Gln Met Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala Glu Trp Asp Arg Leu His  
 Pro Val His Ala Gly Pro Ile Ala Pro Gly Gln Met Arg Glu Pro Arg Gly Ser  
 Asp Ile Ala Gly Thr Thr Ser Thr Leu Gln Glu Gln Ile Gly Trp Met Thr Asn  
 Asn Pro Pro Ile Pro Val Gly Glu Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu  
 Asn Lys Ile Val Arg Met Tyr Ser Pro Thr Ser Ile Leu Asp Ile Arg Gln Gly  
 Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala  
 Glu Gln Ala Ser Gln Glu Val Lys Asn Trp Met Thr Glu Thr Leu Leu Val Gln  
 Asn Ala Asn Pro Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro Ala Ala Thr  
 Leu Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly Pro Gly His Lys Ala  
 Arg Val Leu Ala Glu Ala Met Ser Gln Val Thr Asn Ser Ala Thr Ile Met Met  
 Gln Arg Gly Asn Phe Arg Asn Gln Arg Lys Thr Val Lys Cys Phe Asn Cys Gly  
 Lys Val Gly His Ile Ala Lys Asn Cys Arg Ala Pro Arg Lys Lys Gly Cys Trp  
 Lys Cys Gly Lys Glu Gly His Gln Met Lys Asp Cys Asn Glu Arg Gln Ala Asn  
 Phe Leu Gly Lys Ile Trp Pro Ser His Lys Gly Arg Pro Gly Asn Phe Leu Gln  
 Ser Arg Pro Glu Pro Thr Ala Pro Pro Glu Glu Ser Phe Arg Phe Gly Glu Glu  
 Lys Thr Thr Pro Ser Gln Lys Gln Glu Pro Ile Asp Lys Glu Leu Tyr Pro Leu  
 Ala Ser Leu Arg Ser Leu Phe Gly Asn Asp Pro Ser Ser Gln Met Ala Pro Ile  
 Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys  
 Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys  
 Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr  
 Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu  
 Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu  
 Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Ala  
 Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr  
 Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr  
 Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met  
 Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln  
 Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr  
 Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp  
 Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro

FIGURE 34B

Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val  
Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro  
Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr  
Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu  
Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile  
Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu  
Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr  
Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile  
Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp  
Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe  
Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile  
Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu  
Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr  
Asp Thr Thr Asn Gln Lys Thr Ala Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp  
Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile  
Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln  
Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile  
Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu  
Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn  
Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile  
Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val  
Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu Gly Lys Val  
Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro  
Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp  
Pro Val Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val  
Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn  
Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile  
Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val  
Phe Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly Glu  
Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln  
Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu  
Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln  
Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp  
Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp  
SEQ ID NO: 39



## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/28861

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12N 15/86

US CL : 435/456

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/205.1, 207.1, 227.1, 233.1; 435/69.1, 69.3, 173.3, 235.1, 320.1, 456; 530/23.72;

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
Please See Continuation Sheet

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WO 96/39178 (ERTL et al.) 12 December 1996 (12.12.1996), see page 5, 6, 10, 12, 13 and claims 1 and 5.	1-3, 8-11, 18 ----- 4, 5, 13-17, 29-32, 34, 35, 37
X --- Y	US 6,019,978 A (ERTL et al.) 1 February 2000, (01/02/2000), see columns 2, 7 and 8.	1-3, 8-11, 18 ----- 4, 5, 13-17, 29-32, 34, 35, 37
X,P	US 6,287,571 B1 (ERTL et al.) 11 September 2001 (11/09/2001), see columns 2, 7, 8 and claim 1.	1, 9, 18
X --- Y	US 5,643,579 A (HUNG et al.) 1 July 1997 (01/07/1997), see examples 1, 2, 25 and 26.	1-3, 8, 9-11, 18 ----- 4,5,13-17, 29-32, 34, 35, 37
Y	WANG et al. The use of an E1-deleted, replication-defective adenovirus recombinant expressing the rabies virus glycoprotein for early vaccination of mice against rabies virus. Journal of Virology (March 1997) Vol. 71, No. 5, pp 3677-3683.	1-3, 9-11, 13-18

☒ Further documents are listed in the continuation of Box C.☐ See patent family annex.

## \* Special categories of cited documents:

\*A\* document defining the general state of the art which is not considered to be of particular relevance

\*E\* earlier application or patent published on or after the international filing date

\*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

\*O\* document referring to an oral disclosure, use, exhibition or other means

\*P\* document published prior to the international filing date but later than the priority date claimed

\*T\*

later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\*

document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\*

document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

\*&amp;\*

document member of the same patent family

Date of the actual completion of the international search

06 February 2002 (06.02.2002)

Date of mailing of the international search report

19 AUG 2002

Name and mailing address of the ISA/US

Commissioner of Patents and Trademarks  
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## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/28861

## C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NATUK et al. Immunogenicity of recombinant human adenovirus -human immunodeficiency virus vaccines in chimpanzees. Aids Research and Human Retroviruses (1993) Vol. 9, No. 5, pp395-404, see material and methods.	1, 9, 29-32
Y	PREVEC et al. Immune response to HIV-1 gag antigens induced by recombinant adenovirus vectors in mice and rhesus macaque monkeys. Journal of Acquired Immune Deficiency Syndrome. (1991) Vol. 4, No. 6 pp. 568-76, see abstract.	1, 9, 29-32
Y	LORI et al. Rapid protection against human immunodeficiency virus type 1 (HIV-1) replication mediated by high efficiency non-retroviral delivery of genes interfering with HIV-1 tat and gag. Gene Therapy (1994) Vol. 1, No. 1, pp. 27-31, see abstract.	1, 9
Y	PFARR et al. Differential effects of polyadenylation regions on gene expression in mammalian cells. DNA (1986) Vol. 5, No. 2, pp.115-22, see abstract.	16
Y	NATUK et al. Adenovirus vectored vaccine. Developmental Biological Standards (1994) Vol. 82, pp. 71-77, see abstract.	1, 9

# INTERNATIONAL SEARCH REPORT

International application No.

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## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:  
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-5, 8-11, 13-18, 29-32, 34, 35, 37

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

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**BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING**

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group	Claims	
1	1-5, 8-11, 13-18, 29, 30, 31, 32, 34, 35, 37	The claims are directed to an adenoviral vector that is at least partially deleted of <u><math>\Delta E1</math></u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Gag protein (SEQ ID NO: 29) inserted in the parallel orientation of E1. In addition the vector contains a promoter and a polyadenylation signal.
2	6, 7, 36	The claims are directed to an adenoviral vector that is at least partially deleted of <u><math>\Delta E1</math></u> and <u><math>\Delta E3</math></u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Gag protein (SEQ ID NO: 29).
3	12, 33	The claims are directed to an adenoviral vector that is at least partially deleted of <u><math>\Delta E1</math></u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV protein inserted in the antiparallel orientation of E1.
4	19-23, 38-42	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Gag protein.
5	24, 27, 28, 43, 46, 47	The claim is directed to a method of generating a cellular mediated immune response to HIV Gag protein with the recombinant adenoviral particle.
6	25, 26, 44, 45	The claim is directed to a method of generating a cellular mediated immune response to HIV Gag protein with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.
7	48-51, 53, 54, 56	The claims are directed to an adenoviral vector that is at least partially deleted of <u><math>\Delta E1</math></u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in the parallel orientation of E1.
8	48-51, 53, 54, 56	The claims are directed to an adenoviral vector that is at least partially deleted of <u><math>\Delta E1</math></u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in the parallel orientation of E1.
9	48-51, 53, 54, 56	The claims are directed to an adenoviral vector that is at least partially deleted of <u><math>\Delta E1</math></u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in the parallel orientation of E1.
10	52	The claim is directed to an adenoviral vector that is at least partially deleted of <u><math>\Delta E1</math></u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in the antiparallel orientation of E1.
11	52	The claim is directed to an adenoviral vector that is at least partially deleted of <u><math>\Delta E1</math></u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in the antiparallel orientation of E1.
12	52	The claim is directed to an adenoviral vector that is at least partially deleted of <u><math>\Delta E1</math></u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in the antiparallel orientation of E1.
13	55	The claim is directed to an adenoviral vector that is at least partially deleted of <u><math>\Delta E1</math></u>

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		and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in E1.
14	55	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in E1.
15	55	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in E1.
16	57-61	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Pol protein.
17	62, 65, 66	The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle.
18	63, 64	The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.
19	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in the parallel orientation of E1.
20	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in the parallel orientation of E1.
21	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in the parallel orientation of E1.
22	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in the parallel orientation of E1.
23	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in the antiparallel orientation of E1.
24	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in the antiparallel orientation of E1.
25	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in the antiparallel orientation of E1.
26	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in the antiparallel orientation of E1.
27	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in E1.
28	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in E1.
29	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type

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		adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 13)</u> inserted in E1.
30	74	The claim is directed to an adenoviral vector that is at least partially deleted of <u><math>\Delta</math>E1 and <math>\Delta</math>E3</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 15)</u> inserted in E1.
31	76-80	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Nef protein.
32	81, 84, 85	The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle.
33	82, 83	The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.
34	86a	The claim is drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from three individual vectors.
35	86b, 88, 89	The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from one individual vectors.
36	86c, 88	The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from two individual vectors, one expressing <i>nef-pol</i> fusion and one expressing <i>gag</i> .
37	86d, 87, 88	The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from two individual vectors, one expressing <i>gag-pol</i> fusion and one expressing <i>nef</i> .
38	86e, 88	The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from two individual vectors, one expressing <i>nef-gag</i> fusion and one expressing <i>pol</i> .
39	86f, 88	The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from a single vectors as a fusion protein.
40	86g, 88	The claims are drawn to a multivalent vaccine wherein <i>gag</i> and <i>pol</i> are expressed from two individual vectors.
41	86h, 88, 89	The claims are drawn to a multivalent vaccine wherein <i>gag</i> and <i>pol</i> are expressed individually from one vector.
42	86i, 88	The claims are drawn to a multivalent vaccine wherein <i>pol</i> and <i>nef</i> are expressed from two individual vectors.
43	86j, 88, 89	The claims are drawn to a multivalent vaccine wherein <i>pol</i> and <i>nef</i> are expressed from individually from one vector.
44	86k, 88	The claims are drawn to a multivalent vaccine wherein <i>nef</i> and <i>gag</i> are expressed individually from one vector.
45	86l, 88, 89	The claims are drawn to a multivalent vaccine wherein <i>nef</i> and <i>gag</i> are expressed individually from one vector.
46	86m, 88	The claims are drawn to a multivalent vaccine wherein <i>gag</i> and <i>pol</i> are expressed as a fusion protein from one vector.
47	86n, 88	The claims are drawn to a multivalent vaccine wherein <i>pol</i> and <i>nef</i> are expressed as a fusion protein from one vector.
48	86o, 88	The claims are drawn to a multivalent vaccine wherein <i>nef</i> and <i>gag</i> are expressed as a fusion protein from one vector.

The inventions listed as Groups 1-48 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The technical feature linking groups 1-33 appears to be a recombinant adenoviral vector wherein the adenoviral vector is at least partially deleted in E1 but the vector may contain more deletions, the vector contains wild type sequences including packaging signals and a gene encoding a heterologous HIV protein or fragments thereof. Eril et al. (WO 96/39178) disclose a recombinant adenoviral vector that is deleted in E1 and partially deleted in E3, the remainder of the adenoviral vector contains wild type sequences. The vector additionally contains an insertion of a heterologous protein which includes HIV proteins (see abstract and claims 1 and 5). Therefore, the technical feature linking the inventions of groups 1-45 does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art.

The special technical feature of the following groups 1-3, 7-15, 19-30 and 34-48 is considered to be the combination of sequences that is disclosed in each group, see individual claim groupings above for the different sequences. The DNA disclosed in each group is made up of a different sequence having a different structure and different function.

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The special technical feature of group 4, 16 and 31 is considered to be a method of producing recombinant adenoviral particles. Each group contains different sequences hence the resulting particles would have different structures and functions associated with the particle.

The special technical feature of group 5, 17 and 32 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors. Each group contains different sequences encoding different protein, therefore the resulting immune response will also be different.

The special technical feature of group 6, 18 and 33 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors in conjunction with immunizing the individual a DNA plasmid vaccine. Each method contains different sequences encoding a different protein, therefore the resulting immune response will also be different.

Accordingly, groups 1-48 are not so linked by the same or corresponding technical feature as to form a single general inventive concept.

### Continuation of B. FIELDS SEARCHED Item 3:

WEST 2.0, STN-BIOSIS, MEDLINE

adenoviral vector, deletion, HIV, Gag, polyadenylation signal, CMV promoter

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
21 March 2002 (21.03.2002)

PCT

(10) International Publication Number  
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60/233.180 15 September 2000 (15.09.2000) US
- (71) Applicant (for all designated States except US): **MERCK & CO., INC.** [US/US]: 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).
- (72) Inventors; and
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- (74) Common Representative: **MERCK & CO., INC.**: 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).
- (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
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- with international search report
  - before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments
- (88) Date of publication of the international search report:  
2 May 2002
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: **ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIV-1 GAG, POL, NEF AND MODIFICATIONS**

(57) Abstract: First generation adenoviral vectors and associated recombinant adenovirus-based HIV vaccines which show enhanced stability and growth properties and greater cellular-mediated immunity are described within this specification. These adenoviral vectors are utilized to generate and produce through cell culture various adenoviral-based HIV-1 vaccines which contain HIV-1 gag, HIV-1 pol and/or HIV-1 nef polynucleotide pharmaceutical products, and biologically relevant modifications thereof. These adenovirus vaccines, when directly introduced into living vertebrate tissue, preferably a mammalian host such as a human or a non-human mammal of commercial or domestic veterinary importance, express the HIV-1 Gag, Pol and/or Nef protein or biologically modification thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding HIV-1 Gag, encoding codon optimized HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNase H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The adenoviral vaccines of the present invention, when administered alone or in a combined modality regime, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.

WO 02/22080 A3



# INTERNATIONAL SEARCH REPORT

International application No.

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## A. CLASSIFICATION OF SUBJECT MATTER

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## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/205.1, 207.1, 227.1, 233.1; 435/69.1, 69.3, 173.3, 235.1, 320.1, 456; 530/23.72;

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
Please See Continuation Sheet

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WO 96/39178 (ERTL et al.) 12 December 1996 (12.12.1996), see page 5, 6, 10, 12, 13 and claims 1 and 5.	1-3, 8-11, 18 ----- 4, 5, 13-17, 29, 30, 32, 34, 35, 37
X --- Y	US 6,019,978 A (ERTL et al.) 1 February 2000 (01/02/2000), see columns 2, 7 and 8.	1-3, 8-11, 18 ----- 4, 5, 13-17, 29, 30, 32, 34, 35, 37
X,P	US 6,287,571 A A (ERTL et al.) 11 September 2001 (11/09/2001), see columns 2, 7, 8 and claim 1.	1, 9, 18
X --- Y	US 5,643,579A (HUNG et al.) 1 July 1997 (01/07/1997), see examples 1, 2, 25 and 26.	1-3, 8, 9-11, 18 ----- 4,5,13-17, 29, 30, 32, 34, 35, 37
Y	WANG et al. The use of an E1-deleted, replication -defective adenovirus recombinant expressing the rabies virus glycoprotein for early vaccination of mice against rabies virus. Journal of Virology (March 1997) Vol. 71, No. 5, pp 3677-3683.	1-3, 9-11, 13-18

☒ Further documents are listed in the continuation of Box C.



See patent family annex.

\* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T"

later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X"

document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y"

document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&"

document member of the same patent family

Date of the actual completion of the international search

06 February 2002 (06.02.2002)

Date of mailing of the international search report

13 MAR 2002

Name and mailing address of the ISA/US

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Telephone No. 703-308-0196

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/28861

## C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NATUK et al. Immunogenicity of recombinant human adenovirus -human immunodeficiency virus vaccines in chimpanzees. Aids Research and Human Retroviruses (1993) Vol. 9, No. 5, pp395-404, see material and methods.	1, 9, 29, 30, 32
Y	PREVEC et al. Immune response to HIV-1 gag antigens induced by recombinant adenovirus vectors in mice and rhesus macaque monkeys. Journal of Acquired Immune Deficiency Syndrome. (1991) Vol. 4, No. 6 pp. 568-76, see abstract.	1, 9, 29, 30, 32
Y	LORI et al. Rapid protection against human immunodeficiency virus type 1 (HIV-1) replication mediated by high efficiency non-retroviral delivery of genes interfering with HIV-1 tat and gag. Gene Therapy (1994) Vol. 1, No. 1, pp. 27-31, see abstract.	1, 9
Y	PFARR et al. Differential effects of polyadenylation regions on gene expression in mammalian cells. DNA (1986) Vol. 5, No. 2, pp.115-22, see abstract.	16
Y	NATUK et al. Adenovirus vectored vaccine. Developmental Biological Standards (1994) Vol. 82, pp. 71-77, see abstract.	1, 9

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/28861

## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claim Nos.: 31  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:  
This claim could not be searched because applicant did not provide a CRF.
3. ☐ Claim Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:  
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-5, 8-11, 13-18, 29-32, 34, 35, 37

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

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The special technical feature of group 4, 16 and 31 is considered to be a method of producing recombinant adenoviral particles. Each group contains different sequences hence the resulting particles would have different structures and functions associated with the particle.

The special technical feature of group 5, 17 and 32 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors. Each group contains different sequences encoding different protein, therefore the resulting immune response will also be different.

The special technical feature of group 6, 18 and 33 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors in conjunction with immunizing the individual a DNA plasmid vaccine. Each method contains different sequences encoding a different protein, therefore the resulting immune response will also be different.

Accordingly, groups 1-48 are not so linked by the same or corresponding technical feature as to form a single general inventive concept.

### Continuation of B. FIELDS SEARCHED Item 3:

WEST 2.0, STN-BIOSIS, MEDLINE

adenoviral vector, deletion, HIV, Gag, polyadenylation signal, CMV promoter

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International application No.

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		and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 1)</u> inserted in <u>E1</u> .
14	55	The claim is directed to an adenoviral vector that is at least partially deleted of <u><math>\Delta E1</math></u> and <u><math>\Delta E3</math></u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 5)</u> inserted in <u>E1</u> .
15	55	The claim is directed to an adenoviral vector that is at least partially deleted of <u><math>\Delta E1</math></u> and <u><math>\Delta E3</math></u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 7)</u> inserted in <u>E1</u> .
16	57-61	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Pol protein.
17	62, 65, 66	The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle.
18	63, 64	The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle <u>in addition to</u> administering a DNA plasmid vaccine.
19	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of <u><math>\Delta E1</math></u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 9)</u> inserted in the parallel orientation of <u>E1</u> .
20	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of <u><math>\Delta E1</math></u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 11)</u> inserted in the parallel orientation of <u>E1</u> .
21	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of <u><math>\Delta E1</math></u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 13)</u> inserted in the parallel orientation of <u>E1</u> .
22	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of <u><math>\Delta E1</math></u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 15)</u> inserted in the parallel orientation of <u>E1</u> .
23	71	The claim is directed to an adenoviral vector that is at least partially deleted of <u><math>\Delta E1</math></u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 9)</u> inserted in the antiparallel orientation of <u>E1</u> .
24	71	The claim is directed to an adenoviral vector that is at least partially deleted of <u><math>\Delta E1</math></u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 11)</u> inserted in the antiparallel orientation of <u>E1</u> .
25	71	The claim is directed to an adenoviral vector that is at least partially deleted of <u><math>\Delta E1</math></u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 13)</u> inserted in the antiparallel orientation of <u>E1</u> .
26	71	The claim is directed to an adenoviral vector that is at least partially deleted of <u><math>\Delta E1</math></u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 15)</u> inserted in the antiparallel orientation of <u>E1</u> .
27	74	The claim is directed to an adenoviral vector that is at least partially deleted of <u><math>\Delta E1</math></u> and <u><math>\Delta E3</math></u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 9)</u> inserted in <u>E1</u> .
28	74	The claim is directed to an adenoviral vector that is at least partially deleted of <u><math>\Delta E1</math></u> and <u><math>\Delta E3</math></u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 11)</u> inserted in <u>E1</u> .
29	74	The claim is directed to an adenoviral vector that is at least partially deleted of <u><math>\Delta E1</math></u> and <u><math>\Delta E3</math></u> , the vector contains the cis-acting packaging sequence of the wild type

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		and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in E1.
14	55	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in E1.
15	55	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in E1.
16	57-61	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Pol protein.
17	62, 65, 66	The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle.
18	63, 64	The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.
19	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in the parallel orientation of E1.
20	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in the parallel orientation of E1.
21	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in the parallel orientation of E1.
22	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in the parallel orientation of E1.
23	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in the antiparallel orientation of E1.
24	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in the antiparallel orientation of E1.
25	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in the antiparallel orientation of E1.
26	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in the antiparallel orientation of E1.
27	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in E1.
28	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in E1.
29	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type

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International application No.

PCT/US01/28861

		adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 13)</u> inserted in E1.
30	74	The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1 and ΔE3</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 15)</u> inserted in E1.
31	76-80	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Nef protein.
32	81, 84, 85	The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle.
33	82, 83	The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle <u>in addition to administering a DNA plasmid vaccine.</u>
34	86a	The claim is drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from three individual vectors.
35	86b, 88, 89	The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from one individual vectors.
36	86c, 88	The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from two individual vectors, one expressing <i>nef-pol</i> fusion and one expressing <i>gag</i> .
37	86d, 87, 88	The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from two individual vectors, one expressing <i>gag-pol</i> fusion and one expressing <i>nef</i> .
38	86e, 88	The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from two individual vectors, one expressing <i>nef-gag</i> fusion and one expressing <i>pol</i> .
39	86f, 88	The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from a single vectors as a fusion protein.
40	86g, 88	The claims are drawn to a multivalent vaccine wherein <i>gag</i> and <i>pol</i> are expressed from two individual vectors.
41	86h, 88, 89	The claims are drawn to a multivalent vaccine wherein <i>gag</i> and <i>pol</i> are expressed individually from one vector.
42	86i, 88	The claims are drawn to a multivalent vaccine wherein <i>pol</i> and <i>nef</i> are expressed from two individual vectors.
43	86j, 88, 89	The claims are drawn to a multivalent vaccine wherein <i>pol</i> and <i>nef</i> are expressed from individually from one vector.
44	86k, 88	The claims are drawn to a multivalent vaccine wherein <i>nef</i> and <i>gag</i> are expressed individually from one vector.
45	86l, 88, 89	The claims are drawn to a multivalent vaccine wherein <i>nef</i> and <i>gag</i> are expressed individually from one vector.
46	86m, 88	The claims are drawn to a multivalent vaccine wherein <i>gag</i> and <i>pol</i> are expressed as a fusion protein from one vector.
47	86n, 88	The claims are drawn to a multivalent vaccine wherein <i>pol</i> and <i>nef</i> are expressed as a fusion protein from one vector.
48	86o, 88	The claims are drawn to a multivalent vaccine wherein <i>nef</i> and <i>gag</i> are expressed as a fusion protein from one vector.

The inventions listed as Groups 1-48 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The technical feature linking groups 1-33 appears to be a recombinant adenoviral vector wherein the adenoviral vector is at least partially deleted in E1 but the vector may contain more deletions, the vector contains wild type sequences including packaging signals and a gene encoding a heterologous HIV protein or fragments thereof. Erd et al. (WO 96/39178) disclose a recombinant adenoviral vector that is deleted in E1 and partially deleted in E3, the remainder of the adenoviral vector contains wild type sequences. The vector additionally contains an insertion of a heterologous protein which includes HIV proteins (see abstract and claims 1 and 5). Therefore, the technical feature linking the inventions of groups 1-45 does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art.

The special technical feature of the following groups 1-3, 7-15, 19-30 and 34-48 is considered to be the combination of sequences that is disclosed in each group, see individual claim groupings above for the different sequences. The DNA disclosed in each group is made up of a different sequence having a different structure and different function.

REVISED VERSION

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
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PCT

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(71) Applicant (for all designated States except US): MERCK & CO., INC. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): EMINI, Emilio, A. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). YOUIL, Rima [AU/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). BETT, Andrew, J. [CA/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). CHEN, Ling [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). KASLOW, David, C. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). SHIVER, John, W. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). TONER, Timothy, J. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). CASIMIRO, Daniel, R. [PH/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).

(74) Common Representative: MERCK & CO., INC.; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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Date of publication of the revised international search report:  
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Previous Correction:  
see PCT Gazette No. 30/2002 of 25 July 2002, Section II

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIV1-GAG, POL, NEF AND MODIFICATIONS

(57) Abstract: First generation adenoviral vectors and associated recombinant adenovirus-based HIV vaccines which show enhanced stability and growth properties and greater cellular-mediated immunity are described within this specification. These adenoviral vectors are utilized to generate and produce through cell culture various adenoviral-based HIV-1 vaccines which contain HIV-1 gag, HIV-1 pol and/or HIV-1 nef polynucleotide pharmaceutical products, and biologically relevant modifications thereof. These adenovirus vaccines, when directly introduced into living vertebrate tissue, preferably a mammalian host such as a human or a non-human mammal of commercial or domestic veterinary importance, express the HIV1-Gag, Pol and/or Nef protein or biologically modification thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding HIV-1 Gag, encoding codon optimized HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNase H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The adenoviral vaccines of the present invention, when administered alone or in a combined modality regime, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.

WO 02/022080 A3



## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/28861

<b>A. CLASSIFICATION OF SUBJECT MATTER</b>		
IPC(7) : C12N 15/86		
US CL : 435/456		
According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b>		
Minimum documentation searched (classification system followed by classification symbols)		
U.S. : 424/205.1, 207.1, 227.1, 233.1; 435/69.1, 69.3, 173.3, 235.1, 320.1, 456; 530/23.72;		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)		
Please See Continuation Sheet		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X — Y	WO 96/39178 (ERTL et al.) 12 December 1996 (12.12.1996), see page 5, 6, 10, 12, 13 and claims 1 and 5.	1-3, 8-11, 18 — 4, 5, 13-17, 29-32, 34, 35, 37
X — Y	US 6,019,978 A (ERTL et al.) 1 February 2000, (01/02/2000), see columns 2, 7 and 8.	1-3, 8-11, 18 — 4, 5, 13-17, 29-32, 34, 35, 37
X,P	US 6,287,571 <i>B1</i> (ERTL et al.) 11 September 2001 (11/09/2001), see columns 2, 7, 8 and claim 1.	1, 9, 18
X — Y	US 5,643,579A (HUNG et al.) 1 July 1997 (01/07/1997), see examples 1, 2, 25 and 26.	1-3, 8, 9-11, 18 — 4,5,13-17, 29-32, 34, 35, 37
Y	WANG et al. The use of an E1-deleted, replication -defective adenovirus recombinant expressing the rabies virus glycoprotein for early vaccination of mice against rabies virus. Journal of Virology (March 1997) Vol. 71, No. 5, pp 3677-3683.	1-3, 9-11, 13-18
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* Special categories of cited documents:		
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	
"E" earlier application or patent published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	
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"P" document published prior to the international filing date but later than the priority date claimed		
Date of the actual completion of the international search	Date of mailing of the international search report	
06 February 2002 (06.02.2002)	19 AUG 2002	
Name and mailing address of the ISA/US	Authorized officer	
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## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/28861

## C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NATUK et al. Immunogenicity of recombinant human adenovirus -human immunodeficiency virus vaccines in chimpanzees. Aids Research and Human Retroviruses (1993) Vol. 9, No. 5, pp395-404, see material and methods.	1, 9, 29-32
Y	PREVEC et al. Immune response to HIV-1 gag antigens induced by recombinant adenovirus vectors in mice and rhesus macaque monkeys. Journal of Acquired Immune Deficiency Syndrome. (1991) Vol. 4, No. 6 pp. 568-76, see abstract.	1, 9, 29-32
Y	LORI et al. Rapid protection against human immunodeficiency virus type 1 (HIV-1) replication mediated by high efficiency non-retroviral delivery of genes interfering with HIV-1 tat and gag. Gene Therapy (1994) Vol. 1, No. 1, pp. 27-31, see abstract.	1, 9
Y	PFARR et al. Differential effects of polyadenylation regions on gene expression in mammalian cells. DNA (1986) Vol. 5, No. 2, pp.115-22, see abstract.	16
Y	NATUK et al. Adenovirus vectored vaccine. Developmental Biological Standards (1994) Vol. 82, pp. 71-77, see abstract.	1, 9

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## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:  
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-5, 8-11, 13-18, 29-32, 34, 35, 37

Remark on Protest

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☐

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

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**BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING**

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group	Claims	
1	1-5, 8-11, 13-18, 29, 30, 31, 32, 34, 35, 37	The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Gag protein (SEQ ID NO: 29) inserted in the parallel orientation of E1. In addition the vector contains a promoter and a polyadenylation signal.
2	6, 7, 36	The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> and <u>ΔE3</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Gag protein (SEQ ID NO: 29).
3	12, 33	The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV protein inserted in the antiparallel orientation of E1.
4	19-23, 38-42	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Gag protein.
5	24, 27, 28, 43, 46, 47	The claim is directed to a method of generating a cellular mediated immune response to HIV Gag protein with the recombinant adenoviral particle.
6	25, 26, 44, 45	The claim is directed to a method of generating a cellular mediated immune response to HIV Gag protein with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.
7	48-51, 53, 54, 56	The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in the parallel orientation of E1.
8	48-51, 53, 54, 56	The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in the parallel orientation of E1.
9	48-51, 53, 54, 56	The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in the parallel orientation of E1.
10	52	The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in the antiparallel orientation of E1.
11	52	The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in the antiparallel orientation of E1.
12	52	The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in the antiparallel orientation of E1.
13	55	The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u>

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		and <u>ΔE3</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 1)</u> inserted in E1.
14	55	The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> and <u>ΔE3</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 5)</u> inserted in E1.
15	55	The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> and <u>ΔE3</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 7)</u> inserted in E1.
16	57-61	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Pol protein.
17	62, 65, 66	The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle.
18	63, 64	The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.
19	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 9)</u> inserted in the parallel orientation of E1.
20	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 11)</u> inserted in the parallel orientation of E1.
21	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 13)</u> inserted in the parallel orientation of E1.
22	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 15)</u> inserted in the parallel orientation of E1.
23	71	The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 9)</u> inserted in the antiparallel orientation of E1.
24	71	The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 11)</u> inserted in the antiparallel orientation of E1.
25	71	The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 13)</u> inserted in the antiparallel orientation of E1.
26	71	The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 15)</u> inserted in the antiparallel orientation of E1.
27	74	The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> and <u>ΔE3</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 9)</u> inserted in E1.
28	74	The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> and <u>ΔE3</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 11)</u> inserted in E1.
29	74	The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> and <u>ΔE3</u> , the vector contains the cis-acting packaging sequence of the wild type

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		adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 13)</u> inserted in E1.
30	74	The claim is directed to an adenoviral vector that is at least partially deleted of <u><math>\Delta E1</math> and <math>\Delta E3</math></u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 15)</u> inserted in E1.
31	76-80	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Nef protein.
32	81, 84, 85	The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle.
33	82, 83	The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle <u>in addition to administering a DNA plasmid vaccine.</u>
34	86a	The claim is drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from three individual vectors.
35	86b, 88, 89	The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from one individual vectors.
36	86c, 88	The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from two individual vectors, one expressing <i>nef-pol</i> fusion and one expressing <i>gag</i> .
37	86d, 87, 88	The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from two individual vectors, one expressing <i>gag-pol</i> fusion and one expressing <i>nef</i> .
38	86e, 88	The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from two individual vectors, one expressing <i>nef-gag</i> fusion and one expressing <i>pol</i> .
39	86f, 88	The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from a single vectors as a fusion protein.
40	86g, 88	The claims are drawn to a multivalent vaccine wherein <i>gag</i> and <i>pol</i> are expressed from two individual vectors.
41	86h, 88, 89	The claims are drawn to a multivalent vaccine wherein <i>gag</i> and <i>pol</i> are expressed individually from one vector.
42	86i, 88	The claims are drawn to a multivalent vaccine wherein <i>pol</i> and <i>nef</i> are expressed from two individual vectors.
43	86j, 88, 89	The claims are drawn to a multivalent vaccine wherein <i>pol</i> and <i>nef</i> are expressed from individually from one vector.
44	86k, 88	The claims are drawn to a multivalent vaccine wherein <i>nef</i> and <i>gag</i> are expressed individually from one vector.
45	86l, 88, 89	The claims are drawn to a multivalent vaccine wherein <i>nef</i> and <i>gag</i> are expressed individually from one vector.
46	86m, 88	The claims are drawn to a multivalent vaccine wherein <i>gag</i> and <i>pol</i> are expressed as a fusion protein from one vector.
47	86n, 88	The claims are drawn to a multivalent vaccine wherein <i>pol</i> and <i>nef</i> are expressed as a fusion protein from one vector.
48	86o, 88	The claims are drawn to a multivalent vaccine wherein <i>nef</i> and <i>gag</i> are expressed as a fusion protein from one vector.

The inventions listed as Groups 1-48 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The technical feature linking groups 1-33 appears to be a recombinant adenoviral vector wherein the adenoviral vector is at least partially deleted in E1 but the vector may contain more deletions, the vector contains wild type sequences including packaging signals and a gene encoding a heterologous HIV protein or fragments thereof. Ertl et al. (WO 96/39178) disclose a recombinant adenoviral vector that is deleted in E1 and partially deleted in E3, the remainder of the adenoviral vector contains wild type sequences. The vector additionally contains an insertion of a heterologous protein which includes HIV proteins (see abstract and claims 1 and 5). Therefore, the technical feature linking the inventions of groups 1-45 does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art.

The special technical feature of the following groups 1-3, 7-15, 19-30 and 34-48 is considered to be the combination of sequences that is disclosed in each group, see individual claim groupings above for the different sequences. The DNA disclosed in each group is made up of a different sequence having a different structure and different function.

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The special technical feature of group 4, 16 and 31 is considered to be a method of producing recombinant adenoviral particles. Each group contains different sequences hence the resulting particles would have different structures and functions associated with the particle.

The special technical feature of group 5, 17 and 32 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors. Each group contains different sequences encoding different protein, therefore the resulting immune response will also be different.

The special technical feature of group 6, 18 and 33 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors in conjunction with immunizing the individual with a DNA plasmid vaccine. Each method contains different sequences encoding a different protein, therefore the resulting immune response will also be different.

Accordingly, groups 1-48 are not so linked by the same or corresponding technical feature as to form a single general inventive concept.

### **Continuation of B. FIELDS SEARCHED Item 3:**

WEST 2.0, STN-BIOSIS, MEDLINE

adenoviral vector, deletion, HIV, Gag, polyadenylation signal, CMV promoter